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BLOAT IN CATTLE

XIX. The metabolism of pectin by rumen micro-organisms

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(Received 1 September 1960)

Summary

Red clover (*Trifolium pratense* L.) under New Zealand conditions may contain up to 6.6% pectin. The metabolism of this pectin by rumen micro-organisms has been investigated. Rumen bacteria and protozoa contain pectin methylesterase and polygalacturonase. The galacturonic acid produced by these enzymes is utilised by the organisms with the formation of gas and a mixture of acids, acetic acid being the main acid formed. Gas produced by fermentation of pectin by rumen liquor seems to be due to the action of bacteria rather than of protozoa. The initial rapid gas production from pectin is probably the result of pectin esterase activity in unmasking carboxyl groups.

Fermentation of clover fibre by rumen liquor produced abundant gas, the protozoa having a marked effect on the rate of gas release. The substances in the fibre responsible for the gas formation are not pectic substances alone, but may include the hemicellulose fraction.

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INTRODUCTION

The formation of a stable foam in the rumen seems well established as the cause of bloat in New Zealand cattle grazing red clover. Although leaf protein is probably a major factor contributing to this foam (Mangan 1959), it is evident that other plant factors may be involved.

Several workers have recently speculated on the role of the plant pectic substances in causing bloat (Head 1959; Conrad *et al.* 1958; Pounden and Conrad 1959). Conrad *et al.* examined the pectin content of various bloat-producing feeds and concluded that the pectic substances, together with other cell wall material, were the major plant factors causing this disorder. They found that rumen liquor attacked plant fibre with a rapid release of gas, an effect also observed with a number of carbohydrates, including pectin and its hydrolysis product, the methyl ester of galacturonic acid.

Despite the widespread occurrence of pectin in plants, knowledge of its metabolism by rumen micro-organisms is limited to reports that the protozoon *Ophryoscolex caudatus* can utilise pectin (Williams *et al.* 1960) and that rumen protozoa possess pectin methylesterase and polygalacturonase enzymes (Wright 1960).

Leroy and Michaux (1949) and Michaux (1950, 1951) found that pectic substances were utilised by sheep rumen contents and calculated that about 100 g of pectin was consumed daily by a sheep.

This paper reports observations made on the pectin content of red clover, its breakdown by rumen micro-organisms to galacturonic acid, and the metabolism of galacturonic acid to gaseous and acidic end-products. Studies were also made on gas production by microbial action on plant fibre.

EXPERIMENTAL

Preparations of Suspensions of Bacteria and Protozoa

Samples of rumen liquor, strained through gauze, were obtained from a fistulated cow fed twice daily on fresh red clover or clover hay. This liqour was either diluted with an equal volume of freshly boiled and cooled 0.1M phosphate buffer, pH 6.8, and used immediately (whole rumen liquor), or processed for bacteria or protozoa as described below.

Bacteria. Rumen liquor free of protozoa was obtained by centrifuging at 500 *g* for 5 min. The supernatant was either used immediately (bacterial suspension) or centrifuged at 30,000 *g* for 30 min. The precipitate was twice washed and centrifuged as above in 0.05M phosphate buffer, pH 7.0, containing 0.02% (w/v) $Na_2S.\ 9H_2O$. The bacteria were then suspended in this buffer (washed bacterial suspension) or disrupted in a Nossal shaker (Nossal 1953) to provide a bacterial enzyme extract. The extract was centrifuged at 18,000 *g* for 40 min. at 4°C to remove debris.

Protozoa. Washed suspensions of protozoa from rumen liquor were obtained as previously described (Wright 1960) except that Coleman's buffer was used (Coleman 1958). These suspensions were either used immediately as a mixed suspension, or incubated overnight with antibiotics and mannose to obtain epidinia suspensions or without mannose for holotrich suspensions (Oxford 1958).

Protozoa extracts were prepared by gently grinding thick suspensions of protozoa with Ballotini spheres at 4°c. The extracts were centrifuged at 18,000 *g* for 40 min. at 4°c.

Estimation of Enzyme Activity

Pectin methylesterase and polygalacturonase were measured as previously described (Wright 1960). Gas production by rumen microorganisms was studied in Warburg manometers, using standard techniques (Umbreit *et al.* 1957).

Preparation of Clover Fibres

Three samples of fibre were made, samples 1 and 2 being prepared from the same sample of freshly cut clover. Sample 3 was prepared from clover collected the day after the clover used for the first two samples.

Fibre 1. Clover leaves and stems were placed in boiling ethanol for 5 min. and, after filtering, macerated in a Waring blender with 80% ethanol, and allowed to soak in this solvent for 30 min. at room temperature. The alcohol was filtered off, and the clover was washed with alcohol and ether, then dried at 100°c until the ether had evaporated. The dry fibre was ground in a mill.

Fibre 2. Clover leaves and stems were treated as above, except that hot 80% ethanol was used.

Fibre 3. Clover leaves and stems were crushed in an hydraulic press and the residual fibre centrifuged and washed with water. The fibre was then extracted with alcohol and ether and dried at 100°c.

Analytical Methods

Glucose was estimated by the method of Shaffer and Hartman (1921). Galacturonic acid was assayed with carbazole (Colowick and Kaplan 1957a). The protein content of enzyme extracts was estimated by the Folin reagent (Lowry *et al.* 1951). Organic acids were extracted with ether and separated on celite (Colowick and Kaplan 1957b).

Samples of clover were analysed for pectin by a method similar to that of Kertesz (1951) by preparing clover fibre by method (1) above, then extracting with 0.05N HCl and finally precipitating the pectin as calcium pectate.

The degree of esterification of the pectin was estimated by treating pectin with 0.1N NaOH, steam distilling, and estimating the methanol content of the distillate (Boos 1948). Rumen protozoa were counted using a calibrated chamber.

Pectin. Commercial pectin (Citrus pectin, California Fruit Growers Exchange, California) was purified and low-methoxyl pectin was prepared by the methods described by Jermyn and Tomkins (1950).

RESULTS

Pectin in Red Clover

Several samples of clover were analysed for pectin and its degree of esterification. The results in Table 1 show that the leaves had a higher pectin content and higher degree of esterification than the stems. The level of pectin in all the freshly cut samples ranged from 1.7%–6.6% on a dry weight basis, with a degree of methyl esterification varying between 2.8%–7.2% methoxyl groups by weight. Hydrolysis of the clover pectin and paper chromatography in the solvent system ethyl acetate, pyridine, water (2:1:2 v/v. Jermyn and Isherwood 1949) showed the presence of a strong galacturonic acid spot with small amounts of other sugars whose R_f values corresponded to galactose, glucose, arabinose, and a fast running substance which was probably a pentose. The hydrolysate was run only in one solvent system, so these sugars were not positively identified.

TABLE 1. PECTIN CONTENT OF RED CLOVER

Date Of Collection	Sample	% Pectin	% Esterified With MeOH
16/3/59	Stems	4.9	
	Leaves	6.6	
14/4/59	Stems and leaves (non-bloaty)	4.7	
15/4/59	Stems and leaves (bloaty)	4.5	
11/9/59	Stems and leaves (non-bloaty)	3.0	4.8
21/9/59	Stems and leaves (mild bloat)	1.7	7.2
	Wilted stems and leaves (mild bloat)	1.5	5.9
	Clover hay	1.8	3.5
12/10/59	Stems (bloaty)	5.0	2.8
	Leaves (bloaty)	6.5	4.8
13/10/59	Stems (bloaty)	5.5	3.4
	Leaves (bloaty)	6.3	4.3

DEGRADATION AND FERMENTATION OF PECTIC SUBSTANCES BY RUMEN MICRO-ORGANISMS

Pectic Enzymes

Enzyme extracts from mixed protozoa, epidinia, and rumen bacteria have been examined for the presence of polygalacturonase and pectin methylesterase enzymes. All the extracts contained

both enzymes, the different extracts having similar enzyme activities on a protein content basis.

Formation of Acids from Pectin and Galacturonic Acid

Rumen liquor and washed bacterial suspensions metabolised pectin and galacturonic acid with the formation of acids, 80%–90% being acetic acid. Small amounts of butyric, propionic, and lactic acids were also found.

Rumen liquor was found to utilise glucose more quickly than galacturonic acid. In 4 h 100 ml of rumen liquor used 1.0 g of glucose but only 0.65 g of galacturonic acid. Both the drop in pH and rise in volatile acid production were faster in the case of glucose.

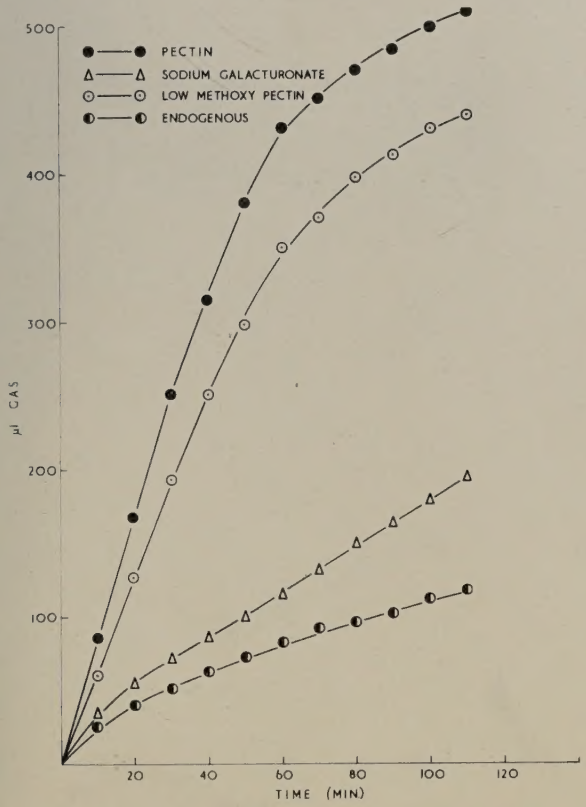

Fig. 1.—Metabolism of pectin by rumen liquor. Each Warburg flask contained 2.5 ml of whole rumen liquor. The side arms contained 0.5 ml of 1% (w/v) substrate neutralised to pH 6.8. Flasks were incubated under O_2-free N_2 at 38°C.

Quantitative experiments showed that rumen bacteria are able to utilise galacturonic acid more rapidly than the protozoa. 100 mg of galacturonic acid were all metabolised by 27 ml of rumen liquor containing only bacteria, while 27 ml of Coleman's buffer containing 1.5×10^6 protozoa (5.3×10^4/ml), 60% of which were epidinia, used only 12.1 mg galacturonic acid. However, the protozoa were in an artificial medium and this may have reduced their metabolic activity. Also the number of protozoa/ml in this sample was only 15%–20% of the average population density of protozoa in the cow sampled (Clarke *pers. comm.*). It is possible that bacteria ingested by the protozoa may have been involved in this utilisation in spite of the presence of antibiotics. However, since a mixed protozoan enzyme preparation was found to utilise galacturonic acid slowly, provided reduced diphosphopyridine nucleotide was present, obviously the protozoa are able to use this substrate.

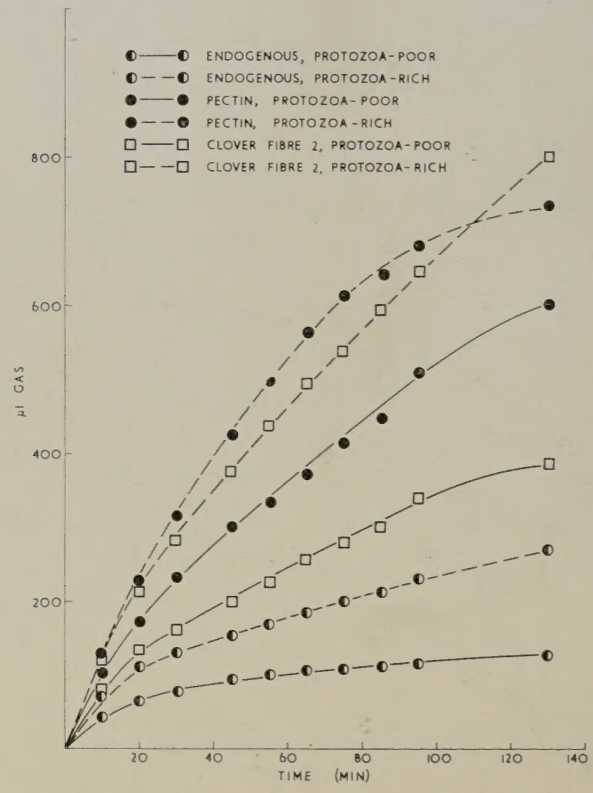

Fig. 2.—Metabolism of pectin by protozoa-poor or protozoa-rich rumen liquor. Samples were incubated as described previously. Each flask contained 4×10^4 or 9×10^4 protozoa.

Gas Production by Rumen Micro-Organisms Metabolising Pectic Substances

The metabolism of pectin and galacturonic acid was studied by following the formation of gas in Warburg manometers. The gas released consisted mainly of carbon dioxide but included gas such as hydrogen formed from endogenous metabolism.

Rumen liquor containing both bacteria and protozoa rapidly attacked pectin and, to a slightly lesser extent, low-methoxy pectin. Surprisingly, galacturonic acid gave rise to much less gas (Fig. 1). After 60 min. the rate of gas production from both samples of pectin had dropped to a rate similar to that from galacturonic acid.

Two samples of rumen liquor, one enriched in protozoa (9×10^4/flask), the other low in protozoa (4×10^4/flask), both

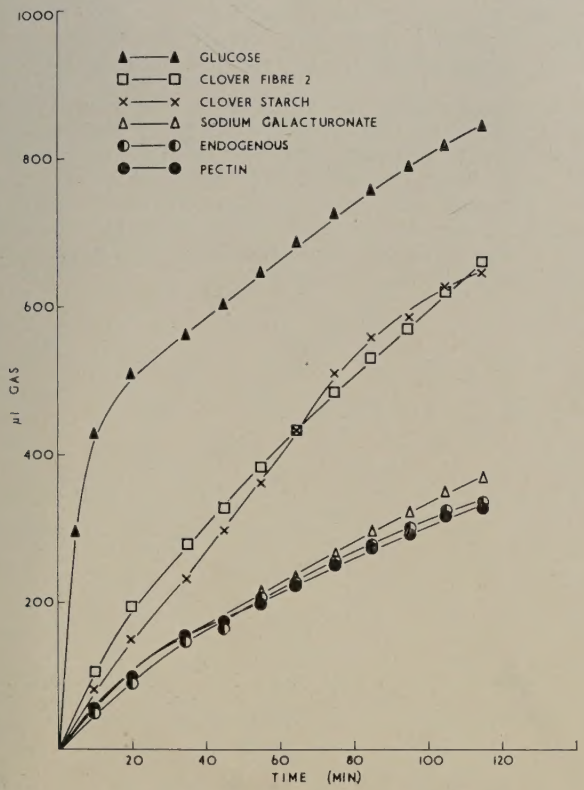

Fig. 3.—Metabolism of pectin by protozoa. Each flask held 2.5 ml of a mixed washed suspension of rumen protozoa containing 1.5×10^6 cells, 33.5% being epidinia. Incubated under O_2-free N_2 at 38°c.

released similar quantities of gas from pectin, 470 μl of gas being formed in each flask in addition to the endogenous gas (Fig. 2).

Since these results suggested that the protozoa do not greatly influence the gas formed from pectin, a washed suspension of protozoa containing epidinia, isotricks, dasytrichs, ostracodinia, diplodinia, entodinia, metadinia, and eudiplodinia, was tested against pectin. Each flask contained 1.5×10^5 cells. It can be seen from Fig. 3 that the gas produced from pectin and galacturonic acid was similar to the endogenous gas and much less than the gas from glucose, clover starch, or clover fibre. Suspensions containing 91% epidinia, while rapidly metabolising glucose, produced only a slow evolution of gas from pectin or galacturonic acid.

Pectin hydrolysed with pectinase was much more rapidly attacked than pectin for the first 20 min., after which it slowed to a rate parallel to that of galacturonic acid. After 80 min. the rates of gas release from hydrolysed pectin, low-methoxy pectin, and galacturonic acid were all similar and less than that from pectin.

This result suggested that some of the gas formed from pectin metabolism arose from the de-esterification of pectin, resulting in the release of carbon dioxide from the rumen liquor concurrently with the unmasking of carboxyl groups. This was tested by incubating a pectin methylesterase extract with citrus pectin, clover pectin, or samples of these de-esterified by treatment with 0.1N NaOH. Fig. 4 shows the release of carbon dioxide from the bicarbonate buffer in this experiment.

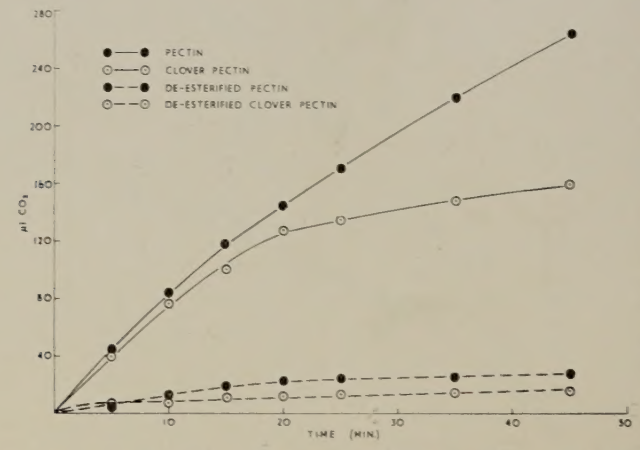

Fig. 4.—Pectin esterase activity. The manometers contained 1.5 ml of 0.018M/$NaHCO_3$, and 0.5 ml of a mixed protozoa enzyme extract. Side arms contained 0.5 ml of 1% neutralised pectin samples or 20 mg of fibre samples. The total volume of liquid in each flask was 2.5 ml. Incubated under 5% (v/v) CO_2 95% (v/v) N_2 at 38°c.

Gas Production by Rumen Micro-Organisms Attacking Clover Fibre

The release of gas from the metabolism of clover fibre by whole rumen liquor or washed protozoa was examined in Warburg flasks. Three samples of fibre (see "Experimental") were incubated with rumen liquor containing bacteria and protozoa. Considerable differences in the rate of gas formation from the different fibres were observed (Fig. 5), fibre 1 giving the greatest gas release, followed by fibres 2 and 3. The gas from pectin was similar to that from fibre 2.

The population of protozoa had a marked effect on the gas formed from fibre as shown in Fig. 2. In this experiment two samples of the same rumen liquor, one of which had been enriched in protozoa, were incubated with pectin or fibre. The protozoa-rich preparation had a higher endogenous gas formation than the

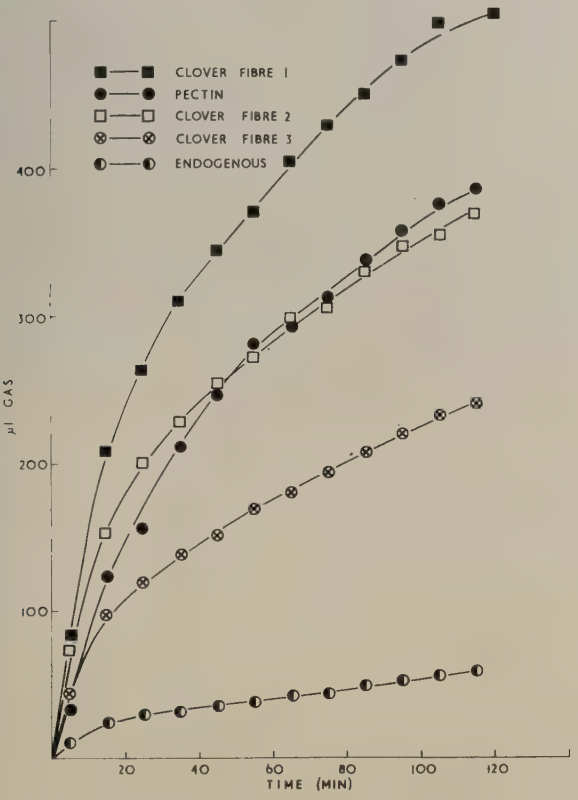

Fig. 5.—Metabolism of clover fibre by rumen liquor. Each flask contained 2.5 ml whole rumen liquor. 40 mg of fibre samples were weighed into side arms.

protozoa-poor suspension. This higher endogenous gas was sufficient to account for the greater quantity of gas from pectin with the protozoa-rich liquor but could not account for the nearly doubled amount of gas arising from metabolism of the fibre.

The results shown in Fig. 3 demonstrate that protozoa do attack fibre with the release of gas. This effect seems to be due to the oligotrich protozoa since a suspension containing only oligotrichs, most of which were epidinia, were found to form gas from fibre at a rate similar to that from clover starch, whereas an holotrich suspension gave little gas from fibre.

A sample of fibre 2 was fractionated into pectin-free fibre and clover pectin. Both were incubated in rumen liquor, the depectinated fibre being attacked with rapid release of gas, while somewhat smaller quantities of gas were produced from citrus pectin, clover pectin, and glucose (Fig. 6). Treating clover fibre with 0.1N NaOH at 39°c for 4 h had a marked effect on the gas produced by rumen liquor. The alkali-treated fibre gave a gas volume barely greater than that of the endogenous gas formed.

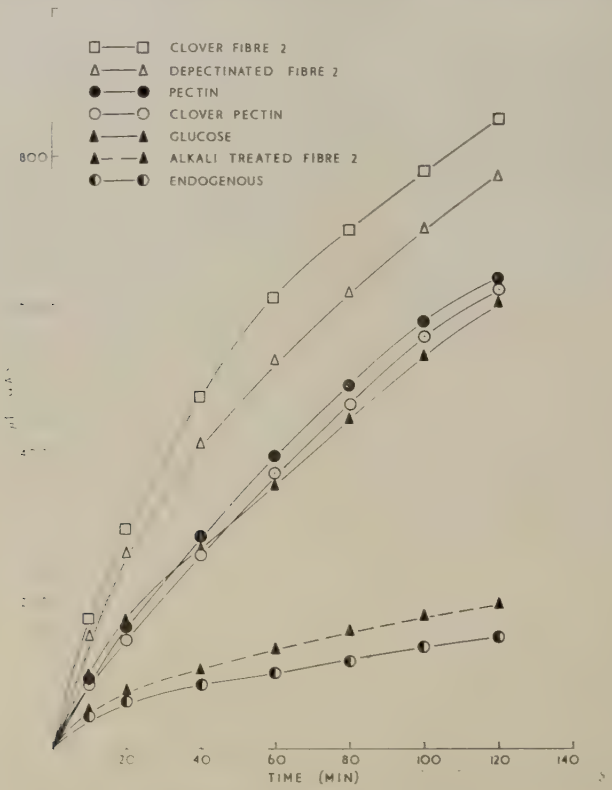

Fig. 6.—Metabolism of pectin by rumen liquor.

Discussion

In studies on gas production in the rumen little previous interest has been taken in the utilisation of plant pectin or plant fibre. The results reported here: (1) support the observations of Conrad *et al.* (1958) that incubating fibre with rumen liquor results in the formation of considerable quantities of gas; but (2) cast doubt on whether plant pectin is the major component responsible.

There can be no doubt that both bacteria and protozoa are able to de-esterify and hydrolyse pectin, and utilise the galacturonic acid formed. The problem of what are the metabolic steps in the breakdown of galacturonic acid has not been examined.

The experiments on gas production from pectin suggest that the bacteria and not the protozoa are responsible for most of the gas formed from this substrate. It is particularly interesting that much more gas is formed from pectin than from galacturonic acid. This result can best be explained on the basis that the rapid initial gas release from pectin arises from its de-esterification to produce free carboxyl groups which liberate carbon dioxide from the bicarbonate normally present in rumen liquor. This view is supported by the slowing down of the rate of evolution of gas until it parallels that from galacturonic acid. As would be expected with such a system, less gas comes from low-methoxy pectin than from pectin itself.

It is apparent that pectin is not the only fermentable substrate in fibre as shown by incubating depectinated fibre with rumen liquor. The substances responsible for this gas production have not been determined, but may be in the hemicellulose fraction as suggested by Pounden and Conrad (1959). The effect of alkali treatment on fibre could be the result of either extracting part of this hemicellulose or perhaps further de-esterifying any alkylated glucuronic acids present in the fibre (Pounden and Conrad 1959).

The fact that protozoa do not appreciably ferment pectin but do attack fibre supports the idea that substances other than pectin are the chief gas precursors from these organisms. It should be emphasised, however, that soluble pectin may be metabolised by protozoa differently from fibre pectin since Oxford (1958) has shown that epidinia can utilise starch grains but not soluble starch. Obviously with pectin this would be difficult to demonstrate experimentally.

As demonstrated with the pectin methylesterase enzyme, clover pectin is partly esterified, and it is likely that some of the gas arising from fibre will be the result of de-esterification of pectin contained in the fibre.

Pounden and Conrad (1959) found a marked daily variation in the level of pectin in alfalfa and claimed to have found a positive relation between the incidence of bloat and content of total pectic substances in the feed. The number of clover samples analysed

for pectin in the present study were too few to give significant results, but no striking difference either in the pectin content or its degree of esterification between bloaty and non-bloaty clover was found.

In addition to its proposed function as a gas precursor, pectin has been suggested as a substance capable of causing a foam in the rumen. Pectin can increase foam production in rumen liquor, as has been shown by Leffel and Doetsch (1959); but the concentration they used (2.5%) is obviously quite different from that likely to be found *in vivo*. Head (1959) examined a foaming substance from rumen liquor and concluded that it was carbohydrate in nature and contained those sugars found in pectin and hemicellulose.

The fact that Bailey (1959) was unable to detect any uronic acids amongst his hydrolysed polysaccharide fractions from the rumen liquor of bloating and non-bloating cows makes it seem unlikely that much soluble pectin exists in rumen liquor. However, it must be remembered that his samples were taken 2 h after the beginning of feeding, by which time considerable hydrolysis of pectin may have occurred.

The effect of various concentrations of pectin on the foam formed from plant cytoplasmic protein, which is almost certainly one of the major foaming compounds in liquor from bloating cows (Mangan 1959), obviously needs to be examined by an accurate reproducible method. Mangan's observation that Na^{+} or Ca^{++} ions can markedly increase the strength of protein foams could imply a role for pectin in determining the level of free ions available for the protein-ion complex. A high degree of esterification in pectin would mean the ready availability of ions, thus increasing foam strength and favouring bloat. On the other hand, a low degree of esterification would enable the free carboxyls in pectin to bind Na^{+} and Ca^{++} ions, thus decreasing foam strength. In addition to this effect, the action of pectin esterase on the high-methoxyl pectin would result in the release of carbon dioxide from rumen liquor.

The degree of pectin esterification will depend on the relative rates of methylation and demethylation, Adamson and Adamson (1958) suggesting that the former process is sensitive to metabolic conditions, such as the availability of adenosine-tri-phosphate, while the latter is insensitive to metabolic conditions. Whether changes in pectin metabolism are related to the bloaty nature of clover is at the moment pure speculation, but it would seem possible that the sporadic nature of bloat outbreaks could be influenced by such a process.

Acknowledgments

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BLOAT IN CATTLE

XX. Lipase activity of rumen micro-organisms

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Summary

Rumen micro-organisms can hydrolyse lipids such as olive oil, linseed oil, tributyrin, monostearin, Tween 80, and trihexanoin at varying rates. The addition of penicillin or terramycin to rumen liquor considerably reduces this lipolytic activity. This suggests that the bacteria rather than other micro-organisms are responsible for most of the lipolysis occurring. These results support the hypothesis that part of the effectiveness of penicillin in controlling bloat may be due to a decrease in bacterial degradation of plant lipids, which are thought to act as anti-foaming agents *in vivo*.

Introduction

It is generally accepted that bloat in cattle grazing on legumes arises from the trapping of the fermentation gas in a foaming rumen ingesta. Various theories have been proposed regarding plant factors suspected of causing this foam. Of these, plant saponins (Lindahl *et al.* 1954; Ferguson and Terry 1955), pectic substances (Head 1959; Pounden and Conrad 1959), and plant cytoplasmic protein (Mangan 1959) appear to be most likely agents involved in the formation of the stable foam in the rumen.

Under New Zealand conditions, it is considered that cytoplasmic protein is the major foaming substance in the rumen, since this protein is rapidly released from the plant by chewing. The level of soluble protein found in rumen liquor is sufficient to form a foam of considerable strength (Mangan 1959). Mangan's observations that foams produced from cytoplasmic protein and from rumen liquor were influenced in similar fashion by variations in pH, lend support to this theory. The sporadic nature of bloat outbreaks cannot, however, be explained by variation in amount of cytoplasmic protein, since no correlation between incidence of bloat and protein content of clover has been found (Mangan and Johns 1957). This suggests that other factors are involved, possibly

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including an anti-foaming agent as postulated by Mangan *et al.* (1959). Their experiments on the effect of penicillin on the foaming of rumen liquor led to the proposal that, in the presence of the antibiotic, the level of anti-foaming agents, presumably lipid in nature, remained high because lipids was inhibited.

The present paper describes results of experiments examining the rate of lipid hydrolysis by rumen liquor and microbial lipases.

Experimental

Rumen Liquor Samples

Rumen liquor was obtained from rumen fistulated cows, stall fed on fresh red clover or clover hay. Samples were taken about 30 min after the commencement of feeding, squeezed through muslin into quart milk bottles, stoppered, and immediately transferred to a room maintained at 38°c.

Preparation of Enzyme Extracts

Bacteria. Rumen liquor was centrifuged at 500 *g* for 5 min to remove protozoa and plant debris. The supernatant was centrifuged at 30,000 *g* for 30 min and the precipitated bacteria washed and centrifuged twice in 0.05M phosphate buffer pH 7.0. The pellet of bacteria was disintegrated in a Nossal shaker (Nossal 1953) and the debris removed by centrifugation at 18,000 *g* for 40 min.

Protozoa. Washed suspensions of mixed protozoa were prepared (Oxford 1958) using Coleman's buffer (Coleman 1958). Epidinia were isolated from suspensions by incubating overnight with mannose (Oxford 1958). The suspensions were centrifuged, washed with cold water, and the cells disrupted by gently grinding with Ballotini spheres at 4°c. The preparations were centrifuged at 18,000 *g* for 40 min and the supernatant used for testing lipase activity.

Measurement of Lipid Hydrolysis by Rumen Liquor

Samples of 50 ml of rumen liquor were added to 100 ml conical flasks containing a weighed amount of lipid (ca. 500 mg) and 100 mg glucose. The flasks were gassed with carbon dioxide, stoppered, and incubated at 38°c for 20–24 h. Flasks containing no added lipid were incubated as controls. Duplicate samples which had either been heated to destroy enzyme activity, or incubated at 4°c, were analysed concurrently. The unhydrolysed lipid remaining after incubation was then estimated by the hydroxamic acid method (Allport and Keyser 1957).

The whole incubation mixture was added to 400 ml absolute ethanol-ether (1 : 1) in a 500 ml volumetric flask and brought to the boil. The sample was cooled, made to volume with ethanol-ether, and filtered through Whatman No. 1 paper. A quantity of 0.5 ml of the filtrate was transferred to a test tube containing 2.5 ml of ethanol-ether (3 : 1). A quantity of 0.5 ml of hydroxylamine hydrochloride reagent (2N) was added, followed by 0.5 ml of 3.5N NaOH. After mixing, the tube was stoppered and stood

at room temperature for 20 min. A quantity of 0.6 ml of 3.5 N HCl was added, the tube shaken and 0.5 ml of 10% (w/v) $FeCl_3.6H_2O$ in 0.1N HCl added. The solution was well mixed and the optical density measured in a Bausch and Lomb spectrophotometer at 525mμ against a solvent blank prepared as above. The amount of glyceride was calculated from graphs prepared from standard solutions of the lipid being analysed.

Iodine Numbers

Samples of the filtered aqueous alcohol–ether solutions were evaporated to dryness, taken up in light petroleum (40°c–60°c), filtered, and evaporated to dryness in weighed tubes *in vacuo*. The iodine number was estimated by a micro-Hanus method.

Lipids

Linseed oil, olive oil, triolein, trihexanoin, and tributyrin were used as triglyceride substrates. Other lipids tested were monostearin (99% pure) and Tween 80 (polyoxyethylene sorbitan mono-oleate).

Results

Recovery of Triglycerides Added to Rumen Liquor

Three samples of linseed oil (512 mg, 196 mg, 97 mg) were added to 50 ml samples of rumen liquor. These samples were then analysed for triglyceride content. A sample of 50 ml of rumen liquor was analysed concurrently as a blank. Recoveries of 96%, 97%, and 100% were obtained.

Rate of Hydrolysis of Linseed Oil by Rumen Liquor

This was examined by incubating rumen contents with the triglyceride in a number of flasks which were periodically analysed. The results in Table 1 show that the ester bonds were split slowly, only 31% having disappeared after 22 h. These results are low when compared with some of the subsequent experiments. However, since analyses of duplicate incubation mixture always

TABLE 1. Rate of Hydrolysis of Linseed Oil by Rumen Liquor

Incubation Time (h)	Linseed Oil Added (mg)	Linseed Oil Recovered (mg)	Lipid Hydrolysed (%)
0	487	470	3
2	504	455	10
4	490	438	12
6	508	430	15
22	487	335	31

50 ml rumen liquor + 100 mg glucose incubated with linseed oil at 38°c.

gave similar results, variations in any single experiment are considered to be significant.

Hydrolysis of Different Glycerides

Analysis of rumen liquor incubated with tributyrin, monostearin, olive oil, or linseed oil for 22 hours showed that tributyrin was hydrolysed more rapidly than the other substrates. (See Table 2.)

TABLE 2. Hydrolysis of Glycerides by Rumen Liquor

Glyceride	Lipid Added (mg)	Lipid Recovered (mg)	Lipid Hydrolysed (%)
Linseed oil	505	435	13
Monostearin	500	395	21
Tributyrin	503	260	49
Olive oil	505	430	15

50 ml rumen liquor + 100 mg glucose incubated with lipid at 38°c for 22 h.

The Effect of Penicillin on Lipase Activity of Rumen Liquor

Mangan *et al.* (1959) have shown that the administration of 100,000–500,000 units of penicillin to a cow had a pronounced effect on the foaming of rumen liquor and on the degree of bloat the day after dosing. This experiment was repeated in order to determine the effect of the antibiotic on the lipase action of rumen liquor. Cow No. 294 was dosed orally with 500,000 units of penicillin at 4 p.m., and the following day a sample of rumen liquor was taken at 10 a.m. from this cow, from its identical twin No. 293, and from cow No. 1. These samples were then incubated with linseed oil for 24 hours. A similar series of incubations were made with the *in vitro* addition of penicillin to the incubation mixture.

The results in Table 3 show that the degrees of lipolysis in rumen samples from cows No. 293 and No. 1 were similar, and considerably greater than in the sample from cow No. 294. In the flasks containing added penicillin, however, lipolysis was considerably reduced.

Effect of Type of Feed and of Various Antibiotics on Lipase Activity of Rumen Liquor

The effect of diet on lipolysis was studied by incubating linseed oil with rumen samples taken during a non-bloating period from identical twin cows, one of which was fed on fresh red clover, the other on clover hay. A similar set of samples was studied during a period when the same cows were bloating. The *in vitro* effect of penicillin, neomycin, terramycin, and streptomycin

TABLE 3. EFFECT OF PENICILLIN ON LIPASE ACTIVITY

Cow No.	Additions *in vitro*	Linseed Oil Added (mg)	Linseed Oil Recovered (mg)	Lipid Hydrolysed (%)
294	No penicillin	493	290	41
	Penicillin (5 units/ml)	498	430	14
293	No penicillin	496	175	65
	Penicillin (5 units/ml)	508	400	20
1	No penicillin	505	145	71
	Penicillin (5 units/ml)	497	360	27

Cow No. 294 was dosed with 500,000 units of penicillin by mouth at 4 p.m. Liquor samples were taken the following day at 10 a.m. and 50 ml aliquots were incubated for 24 h with 100 mg glucose and added linseed oil.

on the lipase activity of rumen liquor was also examined. The results are given in Tables 4 and 5. In both experiments the degree of lipolysis was slightly higher with the clover fed animal; whether this is an animal or dietary difference is not known. The samples taken during non-bloating conditions are notable for their very low degree of lipase action when compared with those taken while the cows were bloating. Penicillin and terramycin both decreased lipolysis markedly, while neomycin and streptomycin had little effect. Estimation of the iodine number of the lipids after incubation showed that all the antibiotics reduced hydrogenation of the unsaturated fatty acids to a similar degree.

TABLE 4. EFFECT OF ANTIBIOTICS ON LIPOLYSIS DURING NON-BLOATING PERIOD

Cow No.	Ration	Antibiotic Added	Linseed Oil Added (mg)	Linseed Oil Recovered (mg)	Lipid Hydrolysed (%)
293	Hay	None	500	460	8
294	Clover	None	493	410	17
		Penicillin	494	455	8
		Neomycin	503	430	14
		Terramycin	507	465	8
		Streptomycin	498	420	16

Samples of rumen liquor were taken during period when animals were not bloating. Aliquots of 50 ml + 100 mg glucose were incubated with linseed oil for 22 h at 38°C. A quantity of 0.5 mg of antibiotic was added per flask.

TABLE 5. Effect of Antibiotics on Lipolysis during Bloating Period

Cow No.	Ration	Antibiotic Added	Linseed Oil Added (mg)	Linseed Oil Recovered (mg)	Lipid Hydrolysed (%)	Iodine Number
293	Hay	None	502	285	43	127
294	Clover	None	495	165	67	78
		Penicillin	502	400	20	109
		Neomycin	495	140	72	100
		Terramycin	498	345	31	104
		Streptomycin	506	185	64	106

Samples of rumen liquor were taken just before cows began to bloat. 50 ml aliquots of 50 ml + 100 mg glucose were incubated with linseed oil for 22 h at 38°c. A quantity of 0.5 mg of antibiotics was added per flask.

Lipase Activity of Enzyme Extracts of Rumen Micro-organisms

The level of lipase activity in cell-free extracts of rumen bacteria, mixed rumen protozoa, and epidinia was tested by incubating the enzymes in Warburg manometers containing various lipids. Lipase action was followed by the release of carbon dioxide from the bicarbonate buffer in the flasks. All three types of extract were found to hydrolyse tributyrin rapidly, and to a lesser extent trihexanoin. Little activity was observed against olive oil, linseed oil, or triolein. The addition of cysteine HCl as a sulphydryl agent had no effect.

Estimation of lipase activity by the hydroxamic acid method gave similar results. The extracts hydrolysed tributyrin quickly, Tween 80 to a considerable extent, and olive oil or linseed oil only slightly.

Discussion

It has been well established that rumen micro-organisms can hydrolyse glycerides. Garton and co-workers (Garton *et al.* 1958; Garton *et al.* 1959) found almost complete hydrolysis of linseed oil after 24 h of incubation. Under similar conditions, a much lower degree of glyceride hydrolysis was found by Allen *et al.* (1959), their results varying from 18%–48% hydrolysis of various triglycerides. Similar variations were found in the present paper. Although these may be due in part to variations in experimental procedure, they are doubtless largely caused by microbial population or changes in the lipolytic activity of individual organisms.

The ability of penicillin to decrease lipolysis is marked and supports the view of Mangan *et al.* (1959) that this antibiotic has an effect on the breakdown rate of a lipid anti-foaming agent. It is interesting that terramycin lowered glyceride hydrolysis since this anti-

biotic has also been found effective in preventing bloat (Barrentine *et al.* 1956). In contrast, neomycin and streptomycin had little effect on lipase activity, and when tried at this laboratory were not effective in preventing bloat. Johnson *et al.* (1959) found that neomycin seemed to reduce bloat, but they had some doubt about their results.

The fact that penicillin had such a marked effect on glyceride hydrolysis makes it seem probable that the bacteria which are sensitive to this antibiotic and the protozoa which are not, were responsible for lipolysis. Since all the antibiotics tested decreased hydrogenation to similar extents, it is unlikely that the penicillin effect on the anti-foaming properties of rumen liquor is due to its effect in reducing the hydrogenation of unsaturated lipids.

The results of Wallace (1959) suggest that lipids are closely connected with the formation of bubbles in rumen liquor. He found that the surface coating of bubbles was rich in lipids (12%–24%). Wallace also examined a froth fraction taken from foaming rumen contents, analysis of which gave lipid contents ranging from 11.8% to 24.3%. These results, together with those of Mangan (1959), make it seem very likely that the foam in rumen liquor can be influenced by the presence of fatty substances possessing anti-foaming ability.

In addition to plant factors, animal factors, such as variations in the rumen microflora, could be involved in bloat. If fluctuations in microbial lipase activity have any influence, one would expect to find high lipase action in a bloating animal and low activity in a normal animal. This was found to be so in Tables 4 and 5; but, since wide variations were found in the other experiments, these differences may be coincidental.

Despite the ability of rumen micro-organisms to hydrolyse linseed oil, examination of cell-free extracts has shown very low enzyme activities towards long chain glycerides, although they readily split water-soluble esters, such as Tween 80, or short chain glycerides such as tributyrin or trihexanoin.

Caution must be exercised in extending these findings to the case of ingested plant lipids *in vivo*, since chloroplast fat consists largely of galactosyl-glyceryl-fatty acid esters (Benson *et al.* 1959; Weenink 1959). The unusual structure of these lipids may imply enzymes different from those hydrolysing triglycerides. Their molecular structure, consisting of a large hydrophilic group attached to a long chain fatty acid, suggests physical properties different from those of triglycerides.

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BLOAT IN CATTLE

XXI. Variation in lipid content of pasture and rumen liquor

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Summary

The lipid contents of ryegrass, red clover, and white clover have been determined. The total lipid extract has been fractionated into four components.

Similar analyses have been made on the rumen liquor of bloating and non-bloating fistulated cows. The relation between total lipid level and bloating is discussed.

INTRODUCTION

It has been shown (Johns 1954) that bloat in cattle fed on red clover is primarily due to a foaming of the rumen ingesta. Further, bloat thus produced can be successfully treated with anti-foaming agents (Reid and Johns 1957). Plant chloroplasts have been shown (Mangan 1958) to have anti-foaming properties, presumably on account of the lipid associated with them. It was therefore of interest to study the lipid content of plant material and also of the rumen liquor from fistulated cows in order to determine whether the concentrations of one or more of the components of these lipids were in any way correlated with the occurrence of bloat in cattle feeding on the pastures from which the lipids were extracted. Lipid content of the fibre material, which was separated from the rumen liquor, was not determined. It was considered that in order to be effective as a foam-preventing agent, the lipid should be able to modify the surface properties of the rumen liquor in some way, and the lipid bound to crude fibre was presumed to be unavailable for this purpose.

MATERIALS AND METHODS

Plant Material

Six separate plants of ryegrass, red clover, and white clover growing in the field were selected and sampled periodically. In the case of both clover species, some plants deteriorated during the

period, and other plants in the same block had to be substituted. Sampling dates were approximately two months apart.

Rumen Material

Several feeding trials were conducted with two fistulated cows used for bloat research at Plant Chemistry Division, D.S.I.R., Palmerston North. The cows were starved overnight and then given similar feed for the first two hours of the trial. Samples of rumen contents were taken at intervals, and the rumen liquor separated from the fibre material, as previously described (Mangan *et al.* 1959), but without the cooling with ice. A suitable volume of the crude rumen liquor was centrifuged at 10,000 *g* for 30 min and the resulting sediment was freeze-dried.

Total Lipid Extraction

Fresh plant material was dried, first in an air oven and then in a vacuum oven. The sediment from the rumen liquor was likewise dried in a vacuum oven. The material was extracted with sodium-dried ether, using the Soxhlet apparatus. Ether was evaporated off on a water bath, and the residue heated in an air oven at 105°c for two hours. This fraction was termed total lipid, although it included any ether-soluble constituents of the plant, not necessarily lipid in nature.

This procedure of solvent removal by evaporation on a water bath and heating in an air oven was used at each stage in the

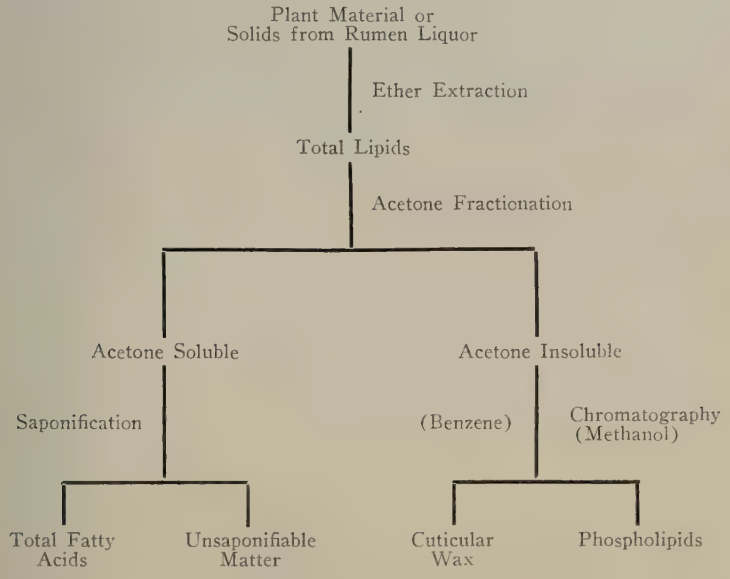

Fig. 1.—Plan of analysis.

analysis set out below, where the weight of a component had to be recorded.

Fractionation of Total Lipids

Fig. 1 summarises the methods used.

The total plant lipids were dissolved in warm acetone. The solution was stored overnight at 4°C, filtered, and the acetone-insoluble material was washed with cold acetone.

Saponification of Acetone-Soluble Fraction

The acetone-soluble fraction was refluxed for one hour with alcoholic KOH. After dilution with water, unsaponifiable material was removed by extracting three times with ether. The combined ether extracts were washed with water to remove all traces of KOH.

The combined aqueous residues from the above extraction were acidified with HCl and extracted three times with ether to remove the total fatty acids fraction. An aqueous NaCl solution was used to remove all traces of HCl from the ether extract.

Chromatography of Acetone-Insoluble Fraction

A silica gel column was prepared, using benzene as solvent. The acetone-insoluble fraction containing cuticular wax and phospholipids was dissolved in hot benzene, cooled to room temperature, and run on to the column, which was then washed with benzene until no further soluble material was found in the eluate. This fraction contained all the cuticular wax. The phospholipids were then quantitatively eluted from the column with methanol (Borgstrom 1952).

Results

Lipid Content of Ryegrass, Red Clover, and White Clover

The means of the total lipid content of samples from six individual plants are given in Table 1, together with standard deviations per plant.

TABLE 1. Total Lipid Content of Individual Pasture Plants (as % dry weight)

Date	Ryegrass	White Clover	Red Clover
May, 1955	6.00 ± .49	4.85 ± .40	5.81 ± .46
July, 1955	5.97 ± .26	4.42 ± .31	4.69 ± .28
September, 1955	5.63 ± .34	4.20 ± .22	5.27 ± .30
November, 1955	5.41 ± .44	5.08 ± .38	5.04 ± .48
January, 1956	5.16 ± .33	4.44 ± .20	3.66 ± .41

The lipids from both the clover species were fractionated by the methods described. The results are shown in Table 2.

TABLE 2. Lipid Fractions of Red and White Clovers (expressed as % total lipid)

Component	White Clover			Red Clover			
	July	Sept.	Feb.	July	Sept.	Nov.	Jan.
Unsaponifiable matter	18.3	19.3	18.8	18.9	21.2	25.9	26.9
Total Fatty acids	42.8	41.3	45.9	36.9	39.8	43.7	26.1
Cuticular wax	10.5	9.1	7.5	11.8	8.9	11.3	9.0
Phospholipids	5.8	8.3	5.9	5.3	4.2	6.3	5.4

Leaf: Stalk Ratio

As the ratio of leaf to stalk in the clover plants was known to vary over a period of more than one season, it was thought that these variations might account for the wider variations in the lipid content of the clovers as compared with that of the ryegrass.

To investigate this, red clover samples were separated into leaf and stalk and the lipid content determined. The results, shown in Table 3, were obtained from individual plants at approximately the same stage of development.

TABLE 3. Lipid Contents of (A) Leaf and (B) Stalk of Red Clover

Date	Dry Matter %		Lipid Content		Ratio of Lipid in Leaf to Lipid in Stalk on D.W.
	Leaf	Stalk	Leaf % D.W.	Stalk % D.W.	
7.11.55*	21.3	14.8	6.09	2.58	2.4 : 1
15.11.55	19.3	13.0	7.22	3.18	2.3 : 1
15.11.55	25.4	13.9	6.37	2.88	2.2 : 1
16.11.55	22.8	11.1	5.61	2.44	2.3 : 1
16.11.55	18.0	11.4	6.05	2.77	2.2 : 1
17.11.55	16.8	13.2	4.54	2.42	1.9 : 1

* Old plants.

Experiment F

Cows Nos. 293 and 294, which are identical twins, were fed on white clover on two consecutive days. On one day they both bloated, and on the other day they did not. The white clover was sampled each day and analyses carried out. The results are given in Table 4.

TABLE 4. EXPERIMENT F. LIPID FRACTIONS IN WHITE CLOVER
(Producing bloating and non-bloating conditions on two consecutive days)

	No Bloat	Bloat
Lipids as % D.M.	4.25	4.51
Components (as % total lipid):		
Unsaponifiable matter	26.7	25.9
Total fatty acids	46.6	53.5
Cuticular wax }	5.3	3.0
Phospholipids }		

Lipid Content of Rumen Material

A typical analysis of rumen liquor taken from cow No. 48 when not bloating is given in Table 5. This shows a clear difference in nature from lipid extracted from plant tissues.

TABLE 5. FRACTIONATION OF LIPIDS FROM RUMEN LIQUOR OF COW NO. 48 (NON-BLOATING)
(Results expressed as percentage of total lipids)

Component	Time from Commencement of Feeding		
	0 h	1 h	2 h
Unsaponifiable matter	37.4	28.8	31.1
Total fatty acid	30.9	41.5	39.5
Cuticular wax	20.0	16.7	22.7
Phospholipids	10.0	7.0	

Feeding Trials

Several feeding trials were conducted with cows Nos. 294 and 48. Rumen liquor samples were analysed for total lipids at intervals during each trial. Red clover, white clover, and ryegrass were used as feed, and both bloating and non-bloating conditions were observed. The results are set out in Table 6.

DISCUSSION

As an evaluation of the analytical methods used, the recoveries at each stage will be considered. Losses at the acetone fractionation stage were low. For 28 fractions the average loss was 2.5% of the original fat. The losses were due to the difficulty of removing the solvents from the fatty material without producing changes in the nature of the material itself. It was shown that two hours in the air oven at 105°c was sufficient for solvent removal without causing an increase in weight due to oxidation.

TABLE 6. LIPID CONTENT OF RUMEN LIQUOR DURING FEEDING EXPERIMENTS

Experiment	Time of Sampling	Cow No. 294			Cow No. 48		
		Red Clover No Bloat			Red Clover No Bloat		
		D.M.	Lipid		D.M.	Lipid	
A	(hours)	g/100 ml	mg/100 ml	% of D.M.	g/100 ml	mg/100 ml	% of D.M.
	0	2.72	184	6.75	2.14	128	5.96
	1	2.70	240	8.92	2.79	183	6.56
	2	2.66	246	9.28	2.55	198	7.77
	4¼	2.62	242	9.24	2.13	152	7.16
	6	2.81	254	9.04	2.06	159	7.72
B		White Clover No Bloat			White Clover Bloat Grade I		
		D.M.	Lipid		D.M.	Lipid	
		g/100 ml	mg/100 ml	% of D.M.	g/100 ml	mg/100 ml	% of D.M.
	0	2.61	189	7.25	2.68	192	7.17
	1½	3.60	239	6.64	3.29	243	7.40
	2½	3.20	231	7.22	3.84	290	7.55
	5¼	4.37	378	8.66	3.47	325	9.36
	7½	3.03	206	6.79	4.11	301	7.32
C		White Clover Bloat Grade II			White Clover Bloat Grade III		
		D.M.	Lipid		D.M.	Lipid	
		g/100 ml	mg/100 ml	% of D.M.	g/100 ml	mg/100 ml	% of D.M.
	0	4.39	283	6.46	4.76	362	7.61
	1	3.19	217	6.79	2.97	214	7.21
	2	3.02	210	6.96	3.62	269	7.45
	4	3.69	278	7.52	3.60	317	8.82
	6	3.40	291	8.56	2.30	163	7.08
D		Red Clover Bloat Grade II			Red Clover Bloat Grade II–III		
		D.M.	Lipid		D.M.	Lipid	
		g/100 ml	mg/100 ml	% of D.M.	g/100 ml	mg/100 ml	% of D.M.
	0	3.34	264	7.91	2.91	227	7.78
	1	2.11	185	8.79	1.66	118	7.11
	2	2.55	202	7.95	2.40	205	8.54
	4	2.71	268	9.91	2.23	229	10.24
	6	3.80	283	7.45	3.07	284	9.27
E		Ryegrass No Bloat			Ryegrass No Bloat		
		D.M.	Lipid		D.M.	Lipid	
		g/100 ml	mg/100 ml	% of D.M.	g/100 ml	mg/100 ml	% of D.M.
	0	—	—	—	3.64	346	9.51
	1	2.57	193	7.51	3.18	319	10.03
	2	1.26	104	8.24	2.93	273	9.29
	4	2.63	209	7.94	—	—	—
	6	1.80	236	13.12	2.29	256	11.20

Note: Bloat grading is according to Johns (1954).

The apparent losses at the saponification stage were larger. In 15 experiments an average of 14.1% was not recovered in the ether extracts. At the time this work was done (1955), these losses could not be completely accounted for. Recently it has been shown (Weenink 1959) that the acetone-soluble neutral lipids of clover and forage grasses contain about 60% of galactosyl glycerol esters of linolenic acid. After saponification 17% of these esters are water-soluble, thereby accounting for 10% of the acetone-soluble lipids. Glycerol from the triglycerides is equivalent to a further 2%. Thus 12% of the acetone-soluble lipids remain in the aqueous solution after the fatty acids and the unsaponifiable material have been extracted. To confirm this, a volume of the aqueous solution was evaporated to dryness, weighed, ignited in a furnace to remove organic material, allowed to cool, and re-weighed. The loss in weight was almost 12% of the original ether-soluble material.

Losses at the silica gel separation were negligible, averaging 3% over 16 experiments.

Cows very seldom bloat when feeding on ryegrass, whereas when the clovers are used as feed, bloat occurs frequently. It can be seen (Table 1) that the lipid content of the ryegrass was higher than that of the clovers on all occasions. These two facts are consistent with the view that lipids possess anti-foaming properties

There have been no very marked changes in the lipid content of the pastures from season to season. On the occasion when the cows bloated one day and did not on the day before (Table 4) there was no marked difference in the lipids.

Further, there was no marked difference between the lipid levels in the rumen liquor whether the cows bloated or did not. If the analysis of the lipids from the rumen liquor of cow No. 48 (Table 5) is compared with that of either of the clovers (Table 2) it will be seen that partial digestion by the cow has caused the proportion of total fatty acids in the lipid material to decrease, and the proportions of the other three constituents to increase. These figures will represent mainly residual microbiological fat, for the animals were starved overnight in preparation for these experiments.

The methods here described give only the total lipids, and no distinction is made between lipid available to act at the surface, and that which is unavailable, such as microbial fat. Thus microbial lipid is probably less effective than the dispersed lipid from plant cells as an anti-foaming agent. Also the rate of removal of the dispersed lipid is not shown by the results given here, but this will have a marked bearing on the occurrence of bloat. Rumen liquor was examined under the microscope and it was found that some species of protozoa were absorbing plant material. It was strongly suspected that intact chloroplasts were being taken into the protozoa. This was subsequently confirmed (Oxford 1958).

It is probable that the rate of release of lipid from the plant cell, and the subsequent rate of uptake by micro-organisms, will be important in determining the anti-foaming properties of rumen liquor. Any further study of the effects of plant lipids on the occurrence of bloat will require a method which is capable of isolating from the lipids those fractions which have anti-foaming properties, and measuring their concentration changes.

Acknowledgments

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THE EFFECT OF DAILY SEPARATION OF SOWS FROM THEIR LITTERS UPON MILK YIELD, CREEP INTAKE, AND ENERGETIC EFFICIENCY

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Summary

Three experiments are described:—

1. Starting 31–35 days after farrowing, sows were separated from their litters for 12 hours daily. A study was made of the effect of this treatment upon milk yield, creep intake, litter weight gains, and the energetic efficiency of lactation.

2 and 3. Separation was commenced 21 days after farrowing. The data in these two experiments were confined to efficiency estimates.

Separation of sows from their litters increased creep intake slightly in the first experiment and substantially in the second and third. In these latter two experiments the increased efficiency of creep energy conversion by the separated litters offset the reduced lactational efficiency of their dams.

Second-litter separated sows exhibited oestrus five to seven days after separation commenced, while first-litter sows required 13–16 days of separation to induce oestrus.

The separated sows in the second experiment were mated during lactation with no apparent ill effects upon the size of the subsequent litters.

The creep intake of the separated litters in the first and third experiments, expressed as a proportion of the total energy intake, reached a level at the end of the sixth week only slightly below that of normally suckled litters at the end of the eighth week.

It is suggested that separation provides many of the benefits of early weaning without incurring the problems associated with that technique.

INTRODUCTION

The increased feed conversion efficiency obtained through early weaning is due almost entirely to the fact that the earlier the occurrence of weaning the lower is the feed intake of the lactating sow and the higher that of the litter in the form of feed consumed directly from the trough. Since feed consumed directly

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by the litter is converted to liveweight gains approximately twice as efficiently as that fed through the sow, it follows that the greater the proportion of the combined sow and litter feed intake that is consumed by the litter as supplementary feed, the greater is the overall feed conversion efficiency.

Sow nutrition studies at Ruakura Animal Research Station, Department of Agriculture, Hamilton, have shown that there is an inverse relation between the milk energy yield of the sow and the creep energy intake of the litter. It would appear, therefore, that litter intake could be deliberately increased by reducing sow milk energy yield. Experiments have indicated, however, that the milk energy yield can be depressed only when sows are first depleted of all body energy reserves and then fed on submaintenance rations (Smith 1960b). The complete removal of the litter from the sow, as in early weaning, is an obvious and drastic method of reducing milk energy production. As an alternative, in the three experiments described in this paper litters were removed from the sows for a period of 12 hours each day in an attempt to reduce their milk consumption and consequently increase their intake of supplementary feed from the creep.

Materials and Methods

Three experiments were conducted. In the first, the yield and composition of the milk of 10 sows were measured, together with sow and litter feed energy intakes, sow weight changes, and litter weight gains. In the second and third experiments measurements were confined to sow and litter intakes, sow weight changes, and litter weight gains.

First Experiment

Ten second-litter Large White × Berkshire crossbred sows were used. Five of these animals were managed in the normal way, while the remaining five were separated from their litters for 12 hours each day; the treatment commenced when the suckers had started to consume appreciable amounts of supplementary feed from the creep. All sows started the treatment on the same day, the ages of their litters varying from 31 to 35 days. Litter size was adjusted to eight pigs within two days of birth.

The sows and litters were fed a concentrate mixture consisting of three parts of barley meal to one part of meat-meal. The sows were fed at the daily rate of 4 lb per sow and 1 lb per piglet suckled, while the litters were fed the same mixture to appetite from the age of three weks. In addition the litters were offered separated milk to appetite. Litters were weighed and their intakes recorded weekly.

The measurement of sow milk yield was carried out in the following manner. Up to the time that separation commenced, the yields of all sows were recorded over a 12 hour period between 6 a.m. and 6 p.m., one day in each week. For the sows in the control

group this procedure was continued through to weaning. The separated sows were away from their litters from 6 a.m. to 6 p.m. Once the treatment commenced the yields of this group were measured between 6 p.m. and 6 a.m., i.e., the whole of the time that the sows and litters were together in the 24 hour period.

Second and Third Experiments

Ten crossbred sows were used, the same animals being employed in both experiments. The data collected relate to their first- and second-litter performances.

The sows were fed at the daily rate of 4½ lb of meal per sow and 1 lb for each piglet suckled. The separation treatment commenced as each litter reached three weeks of age, the same sows being separated from their litters in both experiments. They were fed apart from their litters so that no confusion between sow intake and litter intake could arise.

Creep feed was offered to the litters when they were 10 days old. At the commencement of the second experiment this feed consisted of a barley meal meat meal mixture which proved so unpalatable that little was eaten even after the separation commenced. This same problem had occurred in the first experiment and was the reason for the delay in the start of the separation treatment until the fifth week. As a result of this experience the creep feed was changd to the more palatable mixture shown in Table 1, the change being made after the first two separated litters were 3½ weeks of age. The starter mixture was fed as meal in the second experiment and pelleted in the third experiment.

TABLE 1.—The Creep Feed Mixtures Used in the Second and Third Experiments

Prestarter (Used between 10 Days and 28 Days)		Starter (Used from 28 Days to 49 Days)
Skim-milk powder	70.0	25.0
Fish-meal	7.5	5.0
Meat-meal	7.5	5.0
Sugar	15.0	5.0
Maize-meal		15.0
Barley-meal		40.0
Bran		5.0
	100.0	100.0

The yield and composition of the milk of the sows in the second and third experiments were not measured. The data presented consist of sow feed energy intakes; the feed energy intakes of the litters from the creep, in total in the second experiment and by weeks in the third experiment; sow weight changes; and litter weight gains.

In the course of the first experiment it was observed that the separated sows exhibited oestrus six to seven days after separation commenced. As a result, it was decided to mate any animals showing oestrus in the second experiment in an attempt to establish pregnancy during lactation. The separated sows were tried for heat every day from the commencement of the treatment until they were successfully mated. In the second experiment the separation treatment for each sow continued only until mating was completed, after which the sows and litters were left together in the normal way. It was hoped that by this method any effect of lactation upon the size of the subsequent litters would be accentuated. In the third experiment separation of the sows from their litters was continued throughout lactation; the sows were tried with the boar to establish the time of onset of oestrus but were not mated.

Estimates of Energetic Efficiency

Two methods have been used to estimate the energetic efficiency of lactation in this study:—

1. The method developed earlier in this series (Smith 1960a), ignoring the fact that separated sows in Experiment 2 were pregnant while lactating.

2. The energy intakes of the separated sows were adjusted to make allowance for the overlapping of gestation and lactation. The method used was to substract the estimated maintenance requirements for that part of the lactation during which the sow was both pregnant and lactating, from the feed energy intake for the whole lactation. The feed requirement for milk production during the period of concurrent pregnancy and lactation was thus debited to lactation, while the maintenance requirement was debited to the subsequent gestation.

Results

Experiment 1

The yield and composition of the milk of the individual sows are shown in Table 2.

The effect of stage of lactation upon the mean yield and composition of milk is shown in Fig. 1.

It is evident from Fig. 1 that the separation of the sows from their litters resulted in a substantial reduction in milk production. The mean yields per sow were: up to the time of separation—330 lb and 309 lb; after separation—416 lb and 265 lb; the consecutive figures refer to the normally suckled and the separated groups respectively.

The effect of separation upon milk composition was to accelerate the normal rise in protein content over the last three weeks, and there was a slight tendency similarly to accelerate the normal reduction in lactose content.

TABLE 2.—THE YIELD AND COMPOSITION OF THE MILK

Sow No.	Milk Yield lb	Fat %	Protein %	Lactose %	Energy Content (Therms)*
Treatment: (1) Not Separated					
172	732	8.0	5.9	4.6	415
173	806	8.1	5.8	4.6	463
174	744	8.2	5.8	4.6	429
175	699	8.7	5.8	4.6	417
176	749	7.7	6.2	4.6	423
Totals	3,730				2,147
Means:					
Per sow	746	8.14	5.88	4.6	429.4
Per piglet	93.2				53.7
Treatment: (2) Separated					
177	624	7.8	5.9	4.7	353
178	513	8.3	6.1	4.6	301
179	569	8.6	5.8	4.7	339
180	560	8.2	5.5	4.7	318
181	602	7.3	6.6	4.6	337
Totals	2,868				1,648
Means:					
Per sow	573.6	8.04	5.98	4.66	329.6
Per piglet	71.4				41.2

* Energy values in therms of gross energy: 1,000 kilocalories = 1 therm.

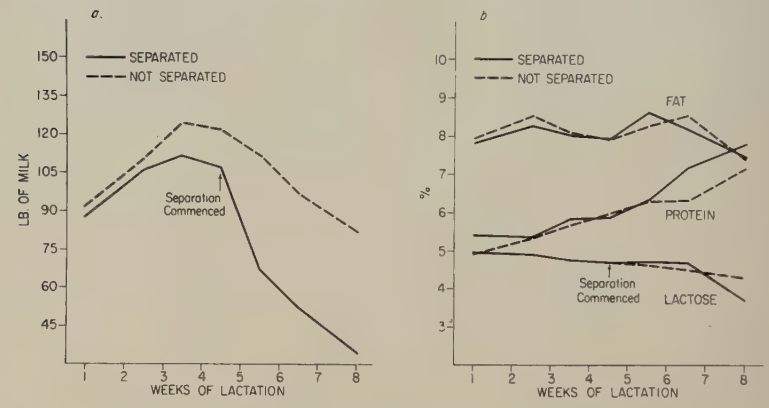

Fig. 1.—The effect of stage of lactation upon mean milk yield and composition.

The Effect of Separation upon Creep Intake

The intakes per pig of (a) sow milk energy and (b) creep feed energy are shown in Table 3.

TABLE 3.—The Mean Intake of Sow Milk and Creep Feed Energy and the Weight Gain per Pig
(Energy values in therms of metabolisable energy)

Treatment	Sow Milk Energy per Pig	Creep Feed Energy per Pig	Total	Gain per Pig (lb)	Therm Intake per lb Gain
Not separated	51	29.7	80.7	30	2.69
Separated	39	32.7	71.7	27	2.66

As shown in Table 3, the litters of the separated group consumed three therms of creep feed energy per pig more than the litters of the non-separated sows. This quantity was much less than expected. The poor response by the separated litters was largely due to the fact that three of the five litters fretted noticeably when away from their dams, and all appeared to find the supplementary feed unpalatable, especially in the early stages of creep feeding.

Efficiency Estimates

Gross efficiency values for the last seven weeks of lactation are shown in Table 4.

TABLE 4.—Gross Efficiency Values for the Last 7 Weeks of Lactation*

Treatment	Efficiency of Milk Production	Efficiency of Lactation**	Efficiency of Energy Conversion by Litters†	Efficiency of Sow Feed Energy Conversion††
Not separated	49.1%	23.8%	41.1	20.0%
Separated	40.2%	22.3%	41.9	17.2%

* Based on formulae published previously (Smith 1960a).

** Therms of energy recovered in litter tissue per 100 therms of sow feed energy intake equivalent and creep feed energy intake by the litter.

† Therms of energy recovered in litter tissue per 100 therms of sow milk and creep feed energy.

†† Therms of energy recovered in the litter per 100 therms of sow feed energy intake equivalent—litter gains and intake from creep feeding excluded.

The comparatively low gross efficiency of milk production exhibited by the separated sows was to be expected in view of their low milk yield and the fact that they were fed the same ration as the non-separated group thoughout lactation. Similarly the efficiency of sow feed energy conversion, i.e., the energy recovery

in the form of litter tissue per 100 therms of sow feed energy intake equivalent (the effect of creep intake upon litter weight gains being excluded), was lower for the separated than for the non-separated group. On the other hand, the estimates of gross efficiency of lactation were not widely different. The reduction in the margin between the values for the two groups in this particular efficiency estimate was due to the fact that the litters

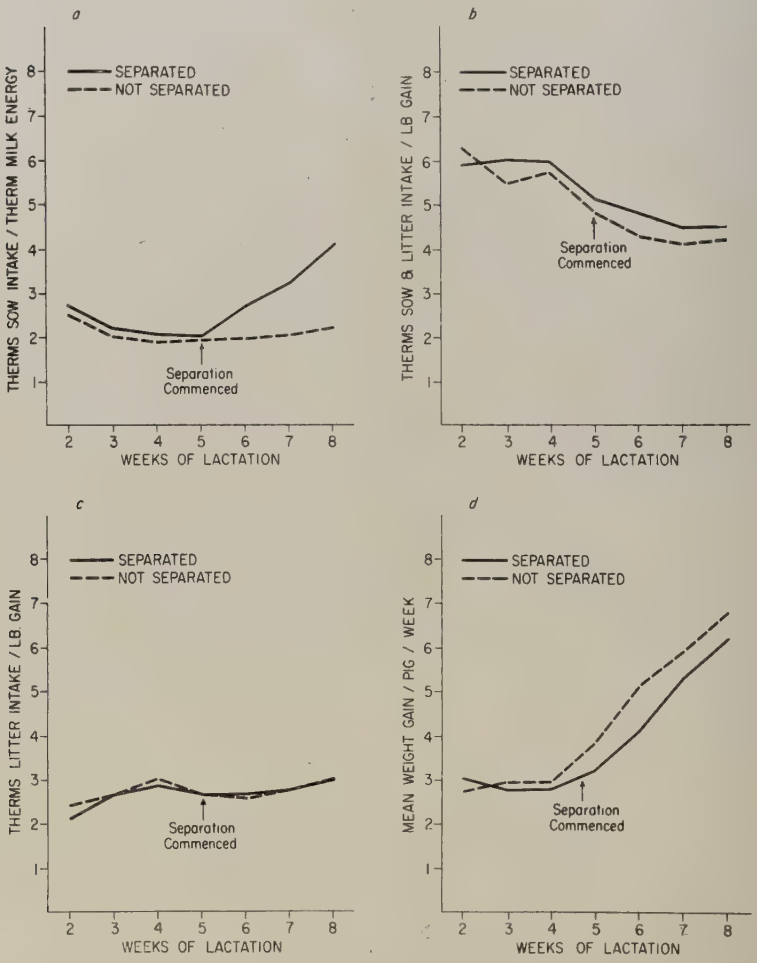

Fig. 2.—(a) The mean sow feed energy intake equivalent per therm of milk energy produced. (b) The mean energy intake per sow and litter per pound of litter gain. (c) The mean energy intake of sow's milk and creep feed by the litter per pound of litter gain. (d) The mean weekly gain per pig.

of the separated sows consumed more creep feed than those of the non-separated group, and used their feed energy slightly more efficiently.

The first three of the four estimates of efficiency appearing in Table 4 are shown graphically on a weekly basis in Fig. 2, with the difference that the calculations are based on the live weight gains of litters rather than the energy content of the gains.

It is at once evident (Fig. 2a) that the efficiency of milk production of the separated sows fell sharply following the start of the treatment in the fifth week. On the other hand (Fig. 2b), the energy requirement in the form of sow feed energy equivalent and creep, per lb of litter gain, shows very little effect following the imposition of the separation treatment. The intake of sow milk and creep feed energy per lb of litter gain was, as indicated in Table 3, substantially similar for the two groups throughout lactation. The growth rate of the litters in the separated group, shown in Fig. 2d, was similar to that of those in the non- separated group up to the beginning of the fifth week; but, once separation commenced, the pigs on this treatment gained between ½ lb and 1 lb per week less than their non-separated mates.

The Second and Third Experiments

The farrowing and rearing data for the 10 sows used in the second and third experiments are shown in Table 5.

In both experiments litter size was adjusted to 10 pigs within 12 hours of birth, or, failing this, an attempt was made to provide an average of 10 pigs per litter within groups. In the second experiment the non-separated group suckled one fewer pig than the

TABLE 5.—Number of Pigs Farrowed and Weaned in the Second and Third Expfriments

Non-Separated					Separated				
	Experiment 2		Experiment 3			Experiment 2		Experiment 3*	
Sow No.	No. of Pigs Born	No. Reared	No. of Pigs Born	No. Reared	Sow No.	No. of Pigs Born	No. Reared	No. of Pigs Born	No. Reared
182	11	11	18	10	187	12	11	17	10
183	9	8	14	10	188	10	10	11	10
184	12	10	12	10	189	12	10	12	10
185	10	10	14	10	190	12	10	15	9
186	15	10	14	10	191	10	9	15	10
Totals	57	49	72	50		56	50	70	49
Means per litter	11.4	9.8	14.4	10.0		11.2	10.0	14.0	9.8

* These litters were conceived during the lactation of experiment 2.

separated, due to the death of one pig at a stage when adjustments to litter size could no longer be made. The same situation arose in regard to the separated group in the third experiment. The data indicate that the litters conceived by the separated sows while lactating during the second experiment were not significantly smaller than those of the non-separated group, the members of which were mated after their litters had been weaned at eight weeks.

Efficiency Estimates

The mean energy intakes of the sows and litters in both groups in the two experiments are shown in Table 6.

TABLE 6.—The Mean Performance Data of the Sows and Litters (Energy Values in Therms)

Wt. at Farrow (lb)	Wt. Loss at Farrow	Wt. at Weaning	Wt. Loss over Lactn. (lb)	Sow Energy Intake Equiv.	Creep Energy Intake	Total Energy Intake	Litter Wt. Gains	Therms Intake lb Gain	% Energy Recovery in Litters
Experiment 2									
Treatment: (1) Non-separated									
382	47	324	11	1,053	328	1,382	365	3.8	29.0
Treatment: (2) Separated									
367	45	317	5	993	423	1,416	381	3.7	29.5
Experiment 3									
Treatment: (1) Non-separated									
386	52	311	23	1,154	495	1,649	412	4.0	27.0
Treatment: (2) Separated									
393	51	314	28	1,028	594	1,622	405	4.0	27.4

The differences between the treatment groups within each experiment shown in the last two columns of Table 6 were not significant. It would appear, therefore, that the reduction in sow milk yield resulting from the separation of sows and litters, and the consequent reduction in efficiency of milk production, were offset by the higher intake and efficiency of conversion of supplementary food by the litters. In the second experiment the separated litters consumed approximately nine therms more creep feed energy per pig than the non-separated litters, while in the third experiment the difference in favour of the separated litters was 11 therms per pig.

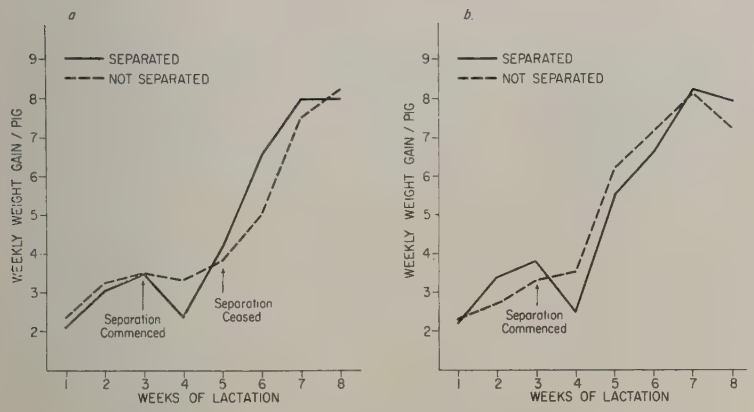

Fig. 3.—The mean weekly gain per pig: (a) in the second experiment; and (b) in the third experiment

The mean weekly weight gains of the litters on the two treatments are shown in Fig. 3.

The separation of the sows and litters at the beginning of the fourth week resulted in a definite depression in growth rate during that week in both experiments. However, there was a complete recovery in the fifth week, by which time all the litters had become reconciled to the absence of the sow and were satisfying their appetites from the creep. During the fourth and fifth weeks of the third experiment, the litters were observed to be waiting impatiently for the return of the sows to the pens at 5 p.m. By the end of the sixth week they evinced no immediate interest in their dams' return and continued to visit the creep for up to an hour before the first post-separation suckling took place.

The Onset of Oestrus

The times of onset of oestrus in the sows in the three experiments are shown in Table 7.

TABLE 7.—Time of Onset of Oestrus Following Separation

Experiment	No. of Sows in Expt.	Age of Litter at First Separation (days)	No. of Sows Showing Oestrus	Days from Separation to Oestrus	Mean
1	5	31–35	4	6–7	6.5
2	5	21	5	13–16	14.0
3	5	21	5	5–6	5.6

The fact that the separated sows in the second experiment were pregnant for the last 21 days of lactation makes possible the adjustment in the total feed energy allowance for lactation, mentioned earlier. When the estimated energy requirements for maintenance over the last three weeks of the suckling period are debited to the gestation requirements of the subsequent litter, the new efficiency estimates are 3.4 therms of sow and litter intake per lb of litter liveweight gain (cf. previous estimate of 3.7) and an energy recovery in the form of litter tissue per 100 therms of sow and litter intake of 32.0 therms (cf. 29.5 therms). If the same adjustments were made in the case of the separated sows in the third experiment on the assumption that they had been mated at the first oestrus after the treatment commenced, a similar increase in efficiency would be found, viz, 3.60 therms per lb of litter gain (cf. 4.0) and 30.4% energy recovery of sow and litter energy intake as litter tissue energy (cf. 27.4).

Discussion

The first experiment described in this paper demonstrated that the milk energy production of sows can be substantially reduced by separating the sows from their litters for a period each day and that, in consequence, the creep intake of the piglets can be increased. The extent of the increase in the intake of creep feed clearly depends upon the palatability of the mixtures used. It is also evident that the reduction in milk energy yield achieved by the separation method results in a drastic lowering of the efficiency of sow milk production. This latter effect is offset by the increased supplementary feed intakes of the litters which are converted to litter weight gains at a relatively high level of efficiency. There are, in addition, two features of the experiments which warrant exploitation.

(1) Mating during Lactation

The first is the finding that when sows are separated from their litters for 12 hours each day they exhibit oestrus and can be successfully mated while still lactating. The period of separation required to ensure oestrus may be quite critical. In trials supplementary to those described above, in which an 8-hour separation period was imposed commencing at 21 days, none of the sows showed any sign of oestrus until the eighth week of lactation. Again, the fact that the second-litter sows used in the first and third experiments exhibited oestrus within a week of the start of separation, while the first-litter animals in the second experiment took approximately one week longer, suggests that the time of onset of oestrus may be conditioned by the age of the sow, or perhaps by the level of milk production, which increases with successive lactations, at least up to the third (Smith 1952). There is obviously need for further investigation of the factors influencing the induction of oestrus in lactating sows.

The establishment of pregnancy during lactation provides both an increase in the energetic efficiency of reproduction, as described earlier, and an increase in the number of litters that can be farrowed per year. Under New Zealand conditions it enables sows farrowed in eary June to be farrowed down again in October instead of December, which is a distinct advantage where a seasonal dairying industry is the source of the bulk of the pig feed used. The saving in time, if mating is achieved in the fourth week, is approximately five weeks, since normally-suckled sows usually go to the boar five to seven days after weaning at eight weeks.

There does appear to be an additional advantage in lactational mating which has not been mentioned previously and which requires further supporting data before it is clearly established. Lodge (1959) has pointed out that sows which do not lose weight during lactation tend to do so immediately after weaning, while those which do lose weight during lactation recover the loss readily after weaning. The sows which were mated during lactation in the second experiment gained 35 lb in the six weeks following weaning, while those mated after weaning lost weight for the first three weeks and added only 5 lb to their weaning weight after six weeks. Both groups were fed similar rations. This loss in weight, which occurs after a normal 8 weeks' weaning and the subsequent remating, requires further investigation to determine the physiologic or metabolic changes which give rise to it; but, whatever these changes may be, they appear either to have been avoided in the case of the sows mated during lactation, or to have occurred during lactation and to have completed their course before weaning.

If the latter were the case it would account for the smallness of the difference in weight loss between the separated and unseparated sows, despite the continued high level of feeding of the former group during the whole period of treatment.

(2) Indifference to Sow at Six Weeks

The second feature of the present experiments which might well be exploited arises from the observed indifference of the litter towards the sow as a source of feed by the time the piglets reached six weeks of age.

It is suggested that methods of rearing pigs other than early weaning, but which yet retain some of the advantages of this system, could well have a place in practical pig keeping. It has been shown (Smith 1960c) that any reduction in the age at which pigs are weaned results in an increase in the energetic efficiency of the reproductive cycle. The limits to the application of this principle and to the exploitation of its full potential appear to be: (a) the incidence of disease, to which younger pigs are generally more susceptible; (b) accommodation standards, which must be higher for younger pigs; and (c) the level of management, which must be highest for the earliest weaned animals. The successful

weaning of normally suckled litters at six weeks is, therefore, possible under a wider range of conditions and management than is weaning at an earlier age; and an examination of the records of the creep intakes of the litters in the present experiments suggests that this should be even more true where separation of the sow and litter has been practised.

The data from the first experiment show that when separation was commenced between 31 and 35 days after farrowing, the separated litters obtained 55% of their feed energy from the creep and 45% from the sow in the sixth week, while the normally-suckled pigs were obtaining 39% and 61% respectively from these two sources at this time. During the seventh week the proportions were 75% from the creep in the case of the separated litters. while the normally suckled pigs obtained 55% from this source.

It is possible to estimate the source of the feed energy of the litters in the third experiment, but not in the second, since weekly creep intakes were not recorded during that experiment. In the third experiment the energy recovery in the form of litter tissue was 27% of the sow and litter feed energy intake, while in the first experiment it was approximately 24%. The sows and litters in the third study were, therefore, slightly more efficient. If the figure of 2.6 therms of sow milk and creep feed energy per lb of litter gain, obtained from the first experiment (Table 3), is applied to the data in the last experiment, the litter gains attributed to creep feed intake will be understated rather than overstated, and the reverse will be true of the estimated milk production.

By applying the above value to the creep intake and weight gain data from the third experiment, estimates of the percentage of the total feed energy derived from the creep were obtained. These are shown in Table 8, together with the data from Experiment 1 and comparable data from 55 litters included in previous sow milk production experiments.

TABLE 8.—Creep Feed Energy Intake Expressed as a % of Total Energy Intake of Litters

Treatment:	Age of Litter in Weeks			
	5	6	7	8
	Percentage			
Experiment No. 1				
(1) Non-separated	16	39	55	70
(2) Separated	14	55	75	85
Experiment No. 3				
(1) Non-Separated	33	44	60	80
(2) Separated	46	65	83	97
55 additional litters				
Non-separated	17	36	56	70

It would appear that early in the seventh week, the separated litters in both the first and third experiments obtained as large a proportion of their total feed requirements from the creep as the non-separated experimental litters and the 55 additional litters were obtaining from that source at eight weeks of age.

These data suggest that separated litters fed palatable creep rations could probably be weaned at six weeks of age with no greater ill effects on growth rate and health than normaly occur after weaning unseparated litters at eight weeks of age. If this is found to be true, the separation technique could contribute materially to increased energetic efficiency of reproduction by: (a) increasing the number of litters produced per sow per year when advantage is taken of the opportunity of mating sows during lactation; (b) decreasing the feed energy requirement per lb of litter gain over lactation by 8% to 10%; (c) increasing efficiency by a further 10% through weaning at six instead of eight weeks of age; and (d) avoiding weight losses which appear to follow the weaning of normally suckled sows.

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THRIPS INFESTATION OF COCKSFOOT SEED CROPS

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(Received 9 November 1960)

Summary

The aim of the investigation was to discover the influence of thrips infestation on the seed yield of cocksfoot in the main seed-producing areas of the South Island of New Zealand. Representative crops were sampled, just prior to harvesting, by collecting ripe seed-heads and storing them until a time convenient for examination. One thousand florets from each crop sample were examined for good seeds, shrivelled seeds, and the number of female thrips present. The number of thrips indicates approximately one-half the number of seeds destroyed, as the female over-winters in the floret where she developed, whereas the male crawls away and does not over-winter.

An analysis of four years' results shows that there is a definite relation between thrips infestation and seed yield. For every 1% increase in infestation there is a corresponding loss in yield of 0.76%. Also, the relation between thrips infestation and good seed for any one district is a measure of the results for any other district in the same season. The number of shrivelled seeds is unrelated to thrips infestation.

Introduction

A study of the thrips fauna of cocksfoot seed crops on the Canterbury Agricultural College farm at Lincoln was carried out during the period 1953 to 1955 by Doull (1956a). This work showed that the thrips fauna was surprisingly large and varied and that one species, *Chirothrips pallidicornis* Preisner (identified first at that time as *C. manicatus* Haliday), was present in numbers sufficiently large to cause appreciable loss in seed yields. As a result this thrips has become known as the "Cocksfoot" thrips. This preliminary investigation by Doull indicated that thrips might be a much more important factor in reducing cocksfoot seed-yields throughout the country than were attacks by the Cocksfoot midge *Stenodiplosis geniculati* var. *dactylidis* (Barnes 1940; Jacks and Cottier 1948) and the cocksfoot stem-borer *Glyphipteryx achlyoessa* Meyrick, previously held to be responsible for much of the loss.

Doull (1956b) studied the biology of the Cocksfoot thrips *C. pallidicornis*, and an account of his findings provides the following information. Adult females over-winter in dead florets of cocksfoot seed-heads. Thrips emerge from their over-wintering quarters in

spring, and may be found on the newly-emerging cocksfoot leaves. There was little evidence to show that any harm was done by thrips feeding during the emergence period or that they fed on floral parts either before or during oviposition. Egg-laying commenced about mid-November when the cocksfoot began to flower. Eggs were laid, normally one to a seed, and placed near the top of the developing ovule. Eggs were never found in unfertilised florets, and, since the centre floret of cocksfoot spikelets is always sterile, no eggs were found in that floret. The larva fed on the developing ovule, and by the time it had reached the pupal stage the seed was completely destroyed. Adults of the new generation were appearing before mid-December. This new generation consists of macropterous females and apterous males. The males leave the florets where they have matured and crawl over the seed-head and, after mating, die. The fertilised females remain in the now-dead floret, where they pass the winter, emerging the following spring to begin a new cycle. Each female thrips represents one seed destroyed. No accurate count of males could be made, but it appeared that the sex ratio was approximately 50:50. A larva that develops into a male destroys one seed in the same manner as a female larva does, hence the number of seeds destroyed should be approximately double the number of female thrips found at harvesting or on examination of a seed sample at a later date.

In order to find out if the conditions obtaining in the crops at Lincoln during this 1953–55 period were representative of the crops in other seed-growing districts and in other seasons it was decided to examine samples from the main seed-growing districts of the South Island over a period of years. This paper deals with the results of those surveys.

Method

The eastern coastal area of Canterbury, Otago, and Southland which produces the bulk of the cocksfoot seed of New Zealand was grouped for survey and analysis purposes into the following 11 well-defined cocksfoot-growing districts:—

No. 1—Rangiora—Kaiapoi.
No. 2—Lincoln—Irwell—Darfield.
No. 3—Methven—Mt. Hutt.
No. 4—Wakanui—Eiffelton—Ashburton—Winchmore.
No. 5—Pleasant Point—Levels—Claremont—Otaio.
No. 6—Five Forks—Kakanui—Palmerston—Dunback.
No. 7—Momona—Taieri—Waitahuna—Balclutha.
No. 8—Kelso—Heriot—Waikaia—Five Rivers—Lumsden.
No. 9—Dipton—Otapiri—Boggyburn—Centre Bush—Winton.
No. 10—Tuatapere—Merrivale—Happy Valley.
No. 11—Fortrose.

Samples were taken, if possible, from representative crops in each district after the crop was judged to be practically ripe, and where possible, just prior to harvesting. A sample consisted of all the seed heads collected from a crop. Each sample was taken

TABLES SHOWING THRIPS INFESTATION OF COCKSFOOT IN THE MAIN SEED-PRODUCING AREAS OF THE SOUTH ISLAND OF NEW ZEALAND FOR FOUR CONSECUTIVE SEASONS FROM 1955–6 TO 1958–9

TABLE 1. SEASON 1955–6

District	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
Number of crops sampled	9	4	4*	14	3	6	8**	4	—	—	—
Percentage of florets containing thrips	8.31	12.05	1.25	16.88	16.43	7.73	0.68	17.03	—	—	—
Percentage of florets containing good seeds	21.07	23.52	10.22	15.75	10.53	20.45	10.4	12.65	—	—	—

TABLE 2. SEASON 1956–7

District	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
Number of crops sampled	14	—	4	12†	7	5	7	4	3	1	1
Percentage of florets containing thrips	7.84	—	0.5	7.5	3.26	6.22	2.63	1.38	5.17	9.0	0.4
Percentage of florets containing good seeds	33.87	—	31.6	32.1	39.11	31.1	38.26	37.03	35.67	35.2	45.0

* Omitted from statistical analysis.
** Six crops omitted from statistical analysis.
† Two crops omitted from statistical analysis.

as uniformly as possible from the whole crop by pulling four seed-heads at equidistant points in each acre of the field. Each sample together with a note of dates, acreage, age, and as much other relevant data about the crop as was available, was wrapped, labelled, and stored for examination during the following winter months. The subsequent examination was carried out by taking a random sample of 1,000 florets obtained by sub-sampling from each seed crop; examining the entire 1,000 with the aid of a low-powered, binocular, stereoscopic microscope; and noting the numbers of: (a) florets containing over-wintering female thrips, (b) good, well-formed seeds, and (c) shrivelled seeds.

Results

The results obtained from the various districts for the four consecutive seasons 1955–6 to 1958–9 inclusive are shown in Tables 1, 2, 3, and 4. For the 1955–6 season, the first year of the survey, the crops in the No. 3 district, Methven–Mt. Hutt, and in the No. 7 district, Momona–Taieri–Waitahuna–Balclutha, were sampled rather too soon for those districts. The result was that a considerable proportion of the florets had not finished flowering; they were thus not in a suitable condition for thrips infestation, and so yielded a larger-than-normal quantity of shrivelled seeds. Again in the 1956–7 season the crops in the Methven–Mt. Hutt district were sampled a little too early; many of the plants were still flowering and a high proportion of shrivelled seeds was again recorded. It soon became apparent that the percentage of shrivelled seeds in a sample bore little, if any, relation to the degree of thrips infestation, but depended almost wholly on the stage of maturity and ripeness of the crop when sampled or on weather conditions prevailing during a critical period of the crop's growth. Because of this, the percentage of shrivelled seeds, although taken into account during the examination of the samples, has been omitted from the tables.

The number of female thrips found in a sample indicates the number of seeds destroyed by female thrips, since one individual insect requires one seed for its development. This, however, must be regarded as approximately half the actual number of seeds destroyed by thrips infestation, for the sex ratio of cocksfoot thrips appears to be in the vicinity of 50:50 and a male also requires one seed for its development. Males are rarely found inside florets in a sample because they leave their feeding ground in the floret and wander over the seed head in search of females which remain where they have matured until the following spring.

Discussion

The relations between good seed, shrivelled seed, and number of thrips were all investigated, and relations within districts were considered separately from relations between districts. Shrivelled seed was unrelated in frequency either to number of good seed

Tables Showing Thrips Infestation of Cocksfoot in the Main Seed-Producing Areas of the South Island of New Zealand for Four Consecutive Seasons from 1955–6 to 1958–9

TABLE 3. Season 1957–8

District	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
Number of crops sampled	12	3	7	11	19	4	7	14	5	—	—
Percentage of florets containing thrips	6.8	3.23	0.43	6.4	1.77	3.85	1.26	1.54	6.98	—	—
Percentage of florets containing good seeds	31.85	40.1	29.2	29.6	35.87	32.52	31.64	28.87	26.52	—	—

TABLE 4. Season 1958–9

District	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
Number of crops sampled	—	—	5	—	3	1	1	4	—	—	—
Percentage of florets containing thrips	—	—	1.86	—	3.97	18.5	8.4	5.3	—		—
Percentage of florets containing good seeds	—	—	32.26	—	31.77	10.7	21.8	22.9	—		—

or number of thrips, except in 1955–6 when there was a weak negative correlation between shrivelled seed and number of thrips. Shrivelled seeds were therefore disregarded in the rest of the investigation.

In all seasons the number of good seeds was negatively correlated to the number of thrips, this correlation being significant at the 0.1% level in all seasons except 1958–9, when it was only significant at the 5% level. In any one season the regression of percentage-good-seed on percentage-thrips was the same in each district, and, further, was equal to the regression between districts. In a single season one regression equation can relate percentage-good-seed and percentage-thrips over the whole of the cocksfoot-seed area of the South Island. The correlations and regressions are shown in Table 5.

TABLE 5. Correlations and Regressions of Percentage Good Seed (G) on Percentage Thrips (T)

Season	Correlation	Regression Equation	No. of Crops
1955–6	— 0.63	G = 25.1 — 0.57T	42
1956–7	— 0.67	G = 40.2 — 0.99T	56
1957–8	— 0.43	G = 34.8 — 0.89T	82
1958–9	— 0.61	G = 32.6 — 1.00T	14

The coefficients of T are not significantly different, so that we may suppose the effect of thrips on good seed to be the same over the four seasons. However, the basic levels of seed production in the four seasons are significantly different, so that we cannot write down one regression equation for the four seasons. The average coefficient of T is — 0.76, and if we use this with the means appropriate to the four seasons we obtain

1955–6 G = 27.5 — 0.76T
1956–7 G = 39.0 — 0.76T
1957–8 G = 34.3 — 0.76T
1958–9 G = 31.4 — 0.76T.

The constant terms in these equations show which seasons produced the most good seed apart from the effect of thrips. It will be noted that the use of an average coefficient of T has not altered the relative positions of the seasons in this respect. The best season was 1956–7, and production declined further in each of the following seasons, but none of these three seasons produced as little seed as the first season 1955–6. Hence it must be concluded that although yields of cocksfoot seed vary greatly from year to year and are almost certainly bound up with the meteorological conditions prevailing throughout a critical period of the crops' growth, yet thrips infestation is responsible for an appreciable loss and the higher the infestation the lower is the yield of good seed.

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THE PERIODICITY OF GERMINATION IN SOME GRASS SPECIES

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Summary

Periodicity of germination was studied in 26 species of grasses.

Considerable variation from species to species was found in rapidity of germination, seasonal periodicity of germination, and length of time during which seed remained viable in the soil.

The following are examples of species showing: (a) no dormancy—*Hordeum murinum* and *Echinochloa crus-galli* var. *frumentacea*; (b) some dormancy—*Festuca rubra* var. *commutata* and *Bromus mollis;* and (c) definite dormancy—*Agrostis tenuis* and *Glyceria fluitans.*

The results are discussed from the viewpoints of: (a) control of weed grasses; and (b) contamination of seed crops by buried viable seeds.

Introduction

Investigations have been made in past years into the dormancy of seeds, particularly those of weeds and legumes (Hyde and Suckling 1953). While dormant grass seeds are mentioned and listed by several authors (Champness and Morris 1958; Milton 1935; Chippindale and Milton 1936), no detailed study has been reported.

It is well known that grass seeds exhibit various degrees of dormancy, and interest has centred mainly upon means of breaking this dormancy to allow all viable seeds to be accounted for in germination tests. Two types of dormancy have been recognised in seeds. "Primary" dormancy has been referred to as that period after the seed is "ripe" but during which no germination occurs even under favourable conditions. The term "after-ripening" is often used in this sense, to cover the period during which physiological changes associated with maturity are taking place; this time lapse varies from species to species. "Secondary" dormancy, on the other hand, occurs when physiologically ripe seeds which have been kept for some time under conditions unfavourable for germination (for example, deep burial in soil) fail to respond when placed in conditions normally conducive to germination. (Crocker and Barton 1953).

The ecological significance of dormancy has been studied only in one or two particular grasses. In the case of wild oats (*Avena fatua* L.) it has been shown (Thurston 1956; Stubbs 1957) that this property accounts in a large measure for their persistency. It would seem reasonable to assume that dormancy of buried seeds in other grasses is of similar significance, and for several reasons the phenomena can be considered of importance. Firstly, seed dormancy may have to be taken into account in choosing methods of combating weed grasses. Secondly, the presence of dormant seed is of importance when selecting land for grass seed crops because contamination must be avoided. Finally, since the period of dormancy determines when germination will occur, it is of interest to know the time range involved in order to foster, or suppress, natural regeneration.

With these factors in mind, sowings of a range of grasses were made to determine when germination occurs in the field under conditions approximating natural dissemination. The present study reports results relating to seeds sown at shallow depths.

Methods

During October and November 1957, that is, before any seeds had been shed in the 1957–8 seeding season, soil samples were taken to a depth of 2 in. from areas where seed was likly to have been shed in the previous (i.e., 1956–7) seeding season. Cores 2 in. long were taken with a cylindrical sampler approximately 1 in. in diameter.

For each sampling, 50 cores were taken from each of a number of areas. The soil samples were brought into the laboratory, and seed recovered by the sieving and flotation process described by Hyde and Suckling (1953). Viability tests on recovered seed were made using the tetrazolium method (Hyde 1956) and by standard germination tests where sufficient seed was available. This technique was adopted in preference to the glass-house technique of counting seedlings of unknown populations of buried seeds as used by Dore and Raymond (1942) and Milton (1943, 1948).

Viable seeds of the following grass species were found: *Lolium perenne* L. (perennial ryegrass), *Poa* spp. *Glyceria fluitans* (L.) R.Br. (floating sweet grass), *Bromus catharticus* Vahl (prairie grass), *Phalaris tuberosa* L. (Toowoomba grass), *Bromus mollis* (L.) (goose-grass), *Hordeum murinum* L. (barley grass), *Alopecurus pratensis* L. (meadow foxtail), *Dactylis glomerata* L. (cocksfoot), *Anthoxanthum odoratum* L. (sweet vernal), *Holcus lanatus* L. (Yorkshire fog), *Paspalum dilatatum* Poir (paspalum), *Danthonia pilosa* R.Br. (danthonia), *Sporobolus capensis* Kunth. (ratstail), and *Eragrostis elongata* (Willd.) (baygrass).

In all species except *Alopecurus pratensis* viability exceeded 50%.

During the 1957–8 seeding season (approximately December 1957 to March 1958) ripe seed was collected from a wide range of grass species in the field. This seed was immediately brought to

the laboratory, cleaned, and viability tested by the tetrazolium method, the same criteria being used for viability in all species as given by Hyde (1956).

Germination tests were then made in specially prepared beds 21 ft × 3 ft containing 6 in. of screened, sterilised soil, the coarser material forming a 3 in. base layer and the finer a 3 in. covering. After consolidation the surface in the beds was about 1 in. below the top of a surround board and 2 in. above ground level.

The grass seed samples from the field harvests were sown in the beds, four replications each of 100 seeds being used for each species. The seeds were placed at roughly equal intervals in a shallow depression across the bed. In view of the wide range of seed size in the present investigation sowing depths were varied from ½ in. for large seeds such as *Bromus catharticus* Vahl. to much less for small seeds such as *Agrostis tenuis* Sibth. Cover was provided by a shallow layer of fine sterilised soil and sand, which also served to make the rows more readily visible during the later progress of the investigation. Seedlings were counted and removed weekly until February 1960, when the investigation was terminated.

Results and Discussion

The course of emergence of the sown species is shown in Table 1, emergence figures after the first four weeks being given for each four-week period only. These figures are the average of the four replicates in each case.

When emergence is expressed as a percentage of viable seeds sown, it will be noted that, of the 26 species used, 20 had an emergence figure of over 50%. There appears to be no relation between these two groups and seed size, or between the groups and periods of "after-ripening".

The annual species show, in general, an emergence of between 90% and 100%, something perhaps not unexpected where survival depends upon establishment from seed each year. However, *Holcus lanatus* (17*), *Festuca rubra* var. *commutata* (15), and *Phalaris tuberosa* (8) also fall within the same emergence group, so that such behaviour is not restricted to annual species.

Two species, *Sporobolus capensis* (25) *and Isachne australis* (26), did not emerge. This might be explained in the case of *Isachne* ("swamp millet") by the well-drained conditions of the raised seed-beds, as it is a grass of wet habitats. The initial germination of the seed (28%) was also low. This explanation does not apply to *Sporobolus,* nor can its failure be attributed to rapid loss of viability, as other tests have shown that seed from the same sample, buried 3 in. deep for over two years, still had a germination of over 40%. It is of interest to observe that in spite of copious seed set, high initial

Text continued on page 258.

* Numbers after species names refer to numbers in Table 1.

TABLE 1.—PROGRESSIVE EMERGENCE OF GRASS SPECIES
(Average of Four Replicates)

No.	Species	Weeks from Sowing: Feb. 1958. 1	2	3	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	Feb. 1960. 64	80	104	Initial Germination %	Total Emergence as % of Viable Seeds Sown
**1	*Hordeum murinum* L. (Barley grass)	37	88	—	89	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	89	86	100
2	*Phleum pratense* L. (Timothy)	0	54	81	83	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	83	93	90
**3	*Echinochloa crus-galli* var. *frumentacea* (Roxb.) Wight. (Japanese millet)	0	9	18	22	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	28	20	100
4	*Alopecurus pratensis* L. (Meadow foxtail)	0	38	39	44	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	47	47	77	61
5	*Danthonia pilosa* R.Br. (Danthonia)	0	11	13	17	28	—	—	—	—	—	29	—	—	—	—	—	—	30	—	—	30	94	32
6	*Lolium multiflorum* Lam. (Italian ryegrass)	0	—	5	46	78	80	—	—	—	—	81	—	—	—	—	—	82	—	—	—	82	86	95
7	*Lolium perenne* L. (Perennial ryegrass)	0	—	2	24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	27	27	42	64
8	*Phalaris tuberosa* L. (Toowoomba grass)	0	—	—	55	89	—	—	—	—	—	—	—	—	—	—	—	—	—	—	90	90	95	95
9	*Festuca arundinacea* Schreb. (Tall fescue)	0	—	—	20	53	—	—	—	—	—	—	—	—	—	—	—	—	55	—	—	55	96	57
10	*Bromus catharticus* Vahl. (Prairie grass)	0	—	—	17	86	93	—	—	—	—	—	—	—	—	—	—	—	—	—	—	93	93	100
11	*Dactylis glomerata* L. (Cocksfoot)	0	—	—	4	42	—	—	—	—	43	—	—	—	—	—	—	44	—	—	—	44	83	53
12	*Cynosurus cristatus* L. (Crested dogstail)	0	—	—	—	48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	51	51	75	68
13	*Anthoxanthum odoratum* L. (Sweet vernal)	0	—	—	—	29	47	—	—	—	—	—	48	—	—	—	—	—	49	—	—	49	61	80

14	*Paspalum urvillei* Steut. (Vasey grass)	0	—	—	—	2	24	29	34	—	—	—	—	—	—	—	—	—	—	—	34	99	35
15	*Festuca rubra* var. *commutata* Gaud. (Chewings fescue)	0	29	65	74	77	—	—	-	—	82	83	86	—	—	—	—	—	-	—	86	85	100
**16	*Bromus mollis* L. (Goose grass)	0	—	—	82	90	—	—	—	—	—	—	—	—	—	92	—	94	97	—	97	100	97
17	*Holcus lanatus* L. (Yorkshire fog)	0		—	21	64	—	—	—	—	—	—	—	—	—	—	—	—	67	—	67	70	96
**18	*Vulpia dertonensis* (All.) Volk. (Hairgrass)	0	—	—	—	30	46	53	54	—	—	—	—	—	—	—	—	56	58	—	58	89	65
**19	*Lagurus ovatus* L. (Harestail)	0	—	—	5	48	—	—	—	—	—	—	—	—	—	—	—	57	—	61	61	87	70
**20	*Poa annua* L. (Poa annua)	0	—	—	—	16	34	35	36	37	—	—	—	—	—	39	—	—	49	-	49	81	60
21	*Agrostis tenuis* Sibth. (Browntop)	0	—	—	—	8	—	—	—	—	—	10	—	—	—	—	11	20	22	23	23	94	24
22	*Glyceria fluitans* (L.) R.Br. (Floating sweet grass)	0	—	—	—	3	—	—	—	—	—	—	—	—	—	—	—	16	17	18	18	94	19
23	*Paspalum dilatatum* Poir. (Paspalum)	0	—	—	—	1	—	—	—	4	5	13	—	15	—	—	—	—	—	—	15	22	68
**24	*Digitaria sanguinalis* (L.) Scop. (Crabgrass)	0	—	—	—	-	—	—		—	—	21	42	47	49	—	—	—	—	—	49	72	68
25	*Sporobolus capensis* Kunth. (Ratstail)	0	—	—	—		—	—	—	—	—	—	—	—	—	—		—	—	—	0	97	0
26	*Isachne australis* R.Br. (Swamp millet)	0	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—			0	28	0

— Denotes no emergence during the period.

* S.E.s of above percentages are $\frac{(\%).\ (100\text{–}\%)}{4G}$ where G is the quoted initial germination percentage; for example, at G = 100% the S.E.s are ± 2.5% at an emergence of 50%, and ± 2.2% at an emergence of 25% (or 75%).

** Annual species.

germination, and good retention of viability, *Sporobolus* plants in the field are usually rather widely dispersed.

Those species showing a period of emergence prolonged over several seasons [for example, *Anthoxanthum odoratum* (13) and *Poa annua* (20)] are the ones most frequently found in soil samples taken for estimates of buried seed populations. (Harris 1959). Other species are also found, but in many cases only in the season when seed is normally shed or comparatively soon thereafter.

Although not evident when collected, the *Lolium perenne* (7) sample used proved to be heavily affected by "blind seed" disease (*Phialea temulenta* Prill and Delacr.) which accounts for the low initial germination (42%). Some 10 months after sowing, four apothecia of *Phialea* were collected from ungerminated seeds in the ryegrass rows. Similarly, the *Alopecurus pratensis* (4) seed used was found to be infested with the foxtail seed midge (*Dasyneura alopecuri* Reut.), and in spite of careful dressing of the seed sample it is probable that some infested seeds were sown, so lowering the emergence figure.

"After-ripening" Period

Any attempt to estimate an after-ripening period from the figures given in Table 1 should be made with caution. It was not always possible to collect seed and sow immediately, although every effort was made to keep the delay to a minimum. Thus it is probable that at least some after-ripening may have occurred before sowing. For example, in the case of *Hordeum murinum* L. (1) seed taken from the plants in a fully ripe state and planted immediately will begin to germinate within a period of 12 hours, indicating a short after-ripening time. On the other hand, *Digitaria sanguinalis* (L.) Scop. (24) showed a lapse of seven months before germination occurred, which would indicate either a prolonged after-ripening time or a period of dormancy.

Secondary Dormancy

The range of species found in field samples taken for buried seed counts, together with the number of viable seeds in such samples, appear to indicate that under some conditions the seeds of several grass species may lie dormant for considerable periods. That some species can, and do, lie dormant for several years is supported by the results of the present study.

From Table 1, the grasses studied may be arranged in three groups according to their germination response in the beds:—

(a) *Those showing no dormancy.*

In this case germination was prompt and nearly complete within 3–4 months from sowing. *Hordeum murinum* (1) and *Phleum pratense* (2) are good examples.

(b) *Those showing some dormancy.*

Here, in spite of early germination of the majority of seeds, a small proportion did not respond until the following spring or autumn, as

illustrated by *Festuca rubra* var. *commutata* (15) and *Bromus mollis* (16).

(c) *Those showing definite dormancy.*

Although several seedlings appeared shortly after sowing, the bulk of the germination did not take place until considerable time had elapsed. *Agrostis tenuis* (21) and *Glyceria fluitans* (22) are examples.

These variations of germination behaviour are of importance when choosing methods of combating weed grasses. Field studies of buried *Hordeum murinum* seeds, for example, have confirmed the behaviour of this species in the present study, and would suggest that control measures applied in the late autumn should be sufficient for complete control if the method used is, in itself, efficient.

On the other hand, a species such as *Poa annua,* where seeds may remain viable for several years and show a seasonal periodicity of germination, will not be controlled by any single treatment, however effective, unless it also accounts for the whole of the buried viable seeds. That this is no small problem is emphasised by the results of buried seed counts, where figures of 300 seeds per sq. ft are not uncommon in samples of pasture soils, and a figure of 11,000 per sq. ft in the top inch of soil has been recorded from a deteriorated sports turf.

In regard to areas for grass-seed crops, perhaps the most important agricultural species involved are the ryegrasses, and the major problem is the contamination of ryegrass areas with other varieties. The results of this experiment indicate that there may be more danger of contamination by perennial ryegrass than by Italian ryegrass, since a higher percentage of perennial ryegrass seed was unaccounted for at the end of the first autumn. Obviously, this suggestion would need further checking before being used as a basis for any fallowing or crop-rotation scheme designed to free an area of land from either *Lolium* species, but it does indicate that the period involved need not be a prolonged one, as would happen if the seed persisted in the soil for several years.

Acknowledgment

The guidance of the late Mr E. O. C. Hyde in the early stages of this study is gratefully acknowledged, as is the technical assistance rendered by Miss M. A. McLeavey.

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VERTICILLIUM WILT OF TOBACCO

IV. A technique for screening tobacco seedlings for resistance to *Verticillium dahliae* Kleb.

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(Received 11 August 1960)

Summary

Liquid cultures of pathogenic strains of *Verticillium dahliae* Kleb. gave highly toxic suspensions which enabled the relative field resistance of tobacco to be determined in the seedling stage.

Aerating the cultures did not increase toxin production, and the wilting-inducing capacity of a culture declined with age.

A technique which promises to be of great assistance in a breeding programme for resistance to verticillium wilt has been evolved and is described.

Introduction

Previous work (McLeod and Thomson 1959) showed that all tobacco varieties and *Nicotiana* spp. tested were susceptible to verticillium wilt, but that several varieties consistently showed less wilt than others when grown in heavily-infested or -inoculated soils. It was also demonstrated that it was possible to obtain wilting of tobacco seedlings by placing them in culture suspensions and culture filtrates of *Verticillium dahliae* Kleb. grown on synthetic liquid media (McLeod and Smith 1961). Efforts were directed to determining the most suitable method of adapting the wilt-inducing capacity of the toxin to differentiate between levels of resistance in tobacco varieties and *Nicotiana* species.

Methods and Results

Seedlings of the varieties under test were raised in nursery flats to the pricking-out stage, lifted carefully, the roots washed free of soil and then immersed in the treatment liquids held in 4 in. × $\frac{5}{8}$ in. or 2 in. × $\frac{1}{2}$ in. test tubes. A minimum of three, usually four, and sometimes six, plants made up a replicate, and three replicates were used for each treatment. Seedlings under test were placed under maximum light conditions in the glass-house which was maintained at above 7° C.

TABLE 1. WILT-INDUCING CAPACITY OF *V. dahliae* CULTURES AS AFFECTED BY AERATION, AGE, AND DILUTION

Variety	Aeration and Flask Cultures												Total	Suspension and Filtrates				Age of Culture				Total
	Aerated						Flask							Full		Half		4 weeks		10 weeks		
	Full		Half		Tenth		Full		Half		Tenth											
	S	F	S	F	S	F	S	F	S	F	S	F		S	F	S	F	Full	Half	Full	Half	
Virginia Gold	27	16	18	8	6	4	30	24	14	12	3	6	168	17	16	1	3	37	12	10	18	114
Harrison's Special	28	17	16	4	5	1	30	18	15	1	4	2	141	15	12	6	2	28	11	2	2	78
T 1 448A														8	4	1	2	17	10	3	5	50
Amarello														8	9	1	0	2	2	0	1	23
Totals	55	33	34	12	11	5	60	42	29	13	7	8		48	41	9	7	84	35	15	26	
Controls distilled water	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	
Controls—sterile Czapek's solution														0	0	0	0					

Figures represent total wilt score, aggregate of three replications.

S = culture suspension.

F = culture filtrates.

Induced Wilting

At intervals commencing four hours after the immersion of the roots in the treatment liquids plants were scored for wilting [0 = no wilt; 1 = slight wilt; 2 = medium wilt (two leaves); 3 = heavy wilt (more than two leaves); 4 = severe wilt (stem limp); 5 = wilted beyond recovery.] Usually the final recording was made after a period of 24 hours, but under conditions of low light intensity some tests ran five days.

Toxin Production

For toxin production modified Czapek's media (0.075% sucrose) was used, either in liquid or agar culture, and stock cultures were maintained on potato dextrose agar.

Suspensions and Filtrates

Culture suspensions were made by maceration of media in a blender for 1½ minutes. Culture filtrates were prepared from suspensions by suction filtration through fine metal gauze, then successively through a single layer of muslin, two layers of muslin, macerated filter paper, filter paper, and double filter paper; the resultant liquor was filtered under pressure through an "Equipk" bacterial filter.

This method of preparing culture filtrates was much more time-consuming, and did not show the increased wilting ability of culture filtrates determined in previous trials (McLeod and Smith 1961) where culture filtrates were prepared by centrifuging. (Table 1.)

Isolates

Several isolates of *V. dahliae*, all ex.-*N. tabacum* Motueka were tested for wilting ability on tobacco seedlings. Provided re-isolation from infected tobacco was carried out periodically, no differences in pathogenicity were found among the various isolates tested.

Age and Dilution

Comparison of suspensions of four-weeks-old and 10-weeks-old flask-grown cultures showed that the wilting capacity of the 10-weeks-old had decreased. Dilution reduced the wilting ability of both the four-weeks-old and the 10-weeks-old cultures (Table 1.)

Aeration

Cultures were aerated by passing compressed air, generated by a diaphragm-type compressor, through a triple-filtered line, in sufficient quantity to produce two bubbles a second in the liquid media. This aeration of cultures showed little difference in wilting capacity of the culture suspension or culture filtrate as compared with flask culture. (Table 1.)

Induced Wilt Varietal Differences

As shown in Table 1, the field-susceptible varieties Harrison's Special and Virginia Gold showed more wilt than the field-resistant varieties T. I. 448A and Amarello. Further tests were carried out to study the reaction of other seedlings. In all, 11 varieties and six species were tested with culture suspensions at different periods. Some of the tests were inconclusive, in that little wilt occurred in any variety, while other tests showed considerable differences in the severity of wilt and the rapidity with which it developed. Using 0 = no wilt and 100 = death in a 24-hour period, the wilt rating of these varieties and species is shown in Table 2.

TABLE 2. Induced Wilt Rating of Seedling Plants Immersed in Culture Suspension of *V. dahliae*

Variety	Wilt Rating	Variety	Wilt Rating
C139	35	Ky 34	61
Amarello	40	DB 101	50
T. I. 448A	40	N. plumbaginifolia	45
Harrison's Special	61	N. paniculata	79
Virginia Gold	70	N. rustica	40
L.O.M.R.	45	N. debneyii	61
L.C.M.R.	45	N. angustifolia	61
B.L.M.R.	45	N. glutinosa	40
Yalomita	55		

With few exceptions, differences shown by varieties in these tests closely parallel their performance in heavily infected fields, showing that by immersion of the roots of seedlings in culture suspensions of *V. dahliae* it is possible to distinguish levels of field resistance which otherwise are shown only at maturity when plants are grown in heavily-inoculated or -infected soil. On field performance *N. rustica* and *N. glutinosa* would not have received the low rating which they obtained in these tests.

The Technique at Present in Use

A start has been made to apply the wilt-inducing capacity of the fungus to tobacco seedlings in a breeding programme for resistance to verticillium wilt. The three varieties which gave the lowest wilt index—Coker 139, T. I. 448A, and Amarello—were tested to see if levels of resistance existed within each variety.

The method adopted is as follows. Seedlings are raised in nursery flats in the glass-house. Approximately 10 days before these seedlings reach the pricking-out stage 500 ml Erlenmeyer flasks containing 150 ml of sterile modified Czapek's solution (0.075% sucrose) are inoculated with *V. dahliae*. The flasks are then placed in a shake flask machine and maintained at a temperature of 20°C ± 5°C. When the plants are ready, 2 in. × ¾ in. test tubes are filled with a culture suspension, the roots of the seedlings carefully washed and then immersed in the suspension under maximum light conditions in the glass-house. Those

plants that show little wilting in four hours are recorded, and 20 hours later the best are transferred to 4 in. pots of sterilised soil, 10 plants per pot. The tubes are topped up with culture suspension, fresh plants inserted, and the procedure repeated. With the equipment available, it is possible to test 1,500 seedlings in each fortnightly period.

The surviving plants from the 4 in. pots are re-potted singly and grown to maturity. During this period these pots are inoculated several times with a macerated mycelial-microsclerotial culture of *V. dahliae* grown on modified Czapek's agar. The progeny of plants which show no wilt, and crosses between them, are being further screened by this technique.

Preliminary results have shown that this technique should overcome the disadvantages of field testing of material. Of the initial 18 Amarello, nine T. I. 448A, and 45 Coker 139 plants selected as possible perental material in the seedling stage, eight Amarello, three T. I. 448A, and 16 Coker 139 plants showed no wilt at maturity.

Of the 77 F1 progenies of crosses between these Amarello, T. I. 448A, and Coker 139 plants, 22 have shown a wilt rating superior to that of any of the perental material.

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CHEMICAL CONTROL OF THE CABBAGE APHID (*Brevicoryne brassicae* L.)

I. Field experiments 1958 and 1960

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(Received 19 December 1960)

Summary

Two experiments in the chemical control of the cabbage aphid are described:

(1) In the first, Metasystox at rates of 4, 6, 8, and 10 oz active ingredient in 30 gal of water per acre applied to leafy swedes gave reduction of the pest significant at the 1% level.

(2) In the second trial, 2 oz active ingredient per acre of Metasystox (i) (Methylisosystox) was the best treatment. At the 1% level it was significantly better than Rogor at 8 oz or PP 175 at 6 oz. These were better than 12 oz of Lebaycid, which was better than 8 oz of Endothion. This last material was better than no treatment at the 5% level only, all material being applied in 20 gal of water to a seed crop of chou moellier in the early spring.

Introduction

The cabbage aphid problem in Canterbury and the control of spring populations on seed-crops with systemic materials have both been outlined by Lowe (1958a, b, 1960).

There is a continuing need to have available tested information on the most economic and effective treatments for control of this pest, and to this end the two trials described herein were undertaken, covering: (1) dosages of Metasystox—the most effective readily available material; and (2) a testing of the lowest effective dosage of this material alongside other materials likely to have some application in this field of pest control.

Experiment 1: Dosage of Metasystox

Experimental Methods and Materials

In the autumn of 1958 a replicated trial of Metasystox at rates of 4, 6, 8, and 10 oz of active ingredient per acre was laid down on a paddock of mature swedes (variety: Calder) at Winchmore,

Ashburton County, New Zealand. Plots of one-fortieth of an acre each were laid out in a randomised block of four replications of each dosage and four untreated controls, and materials were hand-applied in calm weather, using the equivalent of 30 gal of water per acre. Water only was applied to the control plots.

The aphid population was a moderate one, concentrated on the growing point of the plant and the undersides of leaves. The material was applied from immediately above the plants while the operator was walking rapidly between the ridges on which the swedes were growing. Since counts were made on leaves that were mature but not senescent, and on which aphids originally populated the undersides mainly, much of the effect must be regarded as systemic. Counts were made three days after treatment on 20 leaf samples per plot taken at random, and results in Table 1 are based on these counts. Further counts were precluded by natural control, possibly dessication due to high temperatures and low humidity during a period of Föhn winds which followed this count and practically wiped out the whole population of aphids.

Results

Results are set forth in Table 1. In the levels of significance, shown after the manner of Duncan (1955), means designated by the same letter are not significantly different.

TABLE 1. (Experiment 1).—Dosages, Results, and Levels of Significance According to Duncan's Test

Metasystox Dosage (Active Ingredient/ Acre	Mean Log (n + 1) Aphids/20 Leaves	Equivalent Aphids/Leaf	Significance Levels 5%	1%
4 oz	1.34	1.09	a	A
8 oz	1.37	1.17	a	A
10 oz	1.42	1.32	a	A
6 oz	1.45	1.41	a	A
Untreated	2.26 s.d. = .132	9.1	b	B

Conclusions

It is evident from the results that dosages of Metasystox varying from 4 oz to 10 oz active ingredient per acre, when applied in 30 gal of water per acre, give a highly significant reduction of populations of the cabbage aphid

Experiment 2: Various Materials

In view of the efficacy of the lower dosages of Metasystox in Experiment 1, a further experiment, using this material in

comparison with other newer insecticides, was laid down in the spring of 1960 at Broadfields, near Christchurch, on a seed-crop of chou moellier, against the cabbage aphid. The insects were almost totally enclosed in the heads of the plants, having overwintered there, and the experiment was therefore essentially a trial of the systemic properties of the preparations included.

Metasystox had become available as the newer concentrate known commercially as Metasystox (i), being a concentration of the P = O isomer (also known as Methylisosystox in the relevant literature), and it was desired to assess the systemic properties of this preparation at a low dosage, since it was rapidly replacing Metasystox in field use. In an experiment already described (Lowe 1960), as well as in Experiment 1 of this paper, 4 oz active ingredient of Metasystox per acre had been shown to be effective. Because of the circumstances arising in an earlier trial including Rogor (Lowe 1960) it was desirable to repeat the trial, using fresh preparations of this material at the recommended dosage. Lebaycid was included at a higher dosage than used previously because of its likely effectiveness against lepidopterous larvae attacking brassica crops. Endothion had recently been made available, but there appeared to be no record of its effectiveness or otherwise against the cabbage aphid. PP 175 (a Triazinyl-phosphate) had also recently been made available in New Zealand, and it was desirable to test its efficiency against this insect, at recommended rates.

The five treatments and control plots used in this experiment were one-fortieth of an acre in size and replicated six times in a Latin square design. Sampling several days before the experiment was laid down had shown the area to have a mean population of 38 aphids per plant. This figure, when transformed by the same method used in dealing statistically with the results of the experiment, gives a weighted mean of 12 aphids per plant. The most heavily populated area of the paddock was chosen for the experiment, and all materials were hand-applied in ideal conditions in the equivalent of 20 gal water per acre. Chou moellier seed crops are normally treated against cabbage aphid by aerial application of insecticides, and the low gallonage of water applied was chosen as the nearest equivalent that can conveniently be hand-applied. Materials used and the dosage of active ingredient per acre are shown in Table 2.

Results

Counts of total aphids per plant were made at 3, 7, 11, and 26 days after treatment. The whole duration of treatment and counts coincides with a period when alate aphids are almost entirely absent from the population. This fact is reflected in an analysis of variance between days which failed to reveal significant differences in individual treatments, indicating that at this time of the year the population position remains relatively stable between plants. Analysis of variance between treatments was therefore made on all counts. The results are set out according to Duncan's method in

Table 2 which shows that at the 1% level of significance Metasystox (i) is the best treatment applied, Rogor and PP 175 are next but do not differ significantly, and Lebaycid at the dosage used is also significantly better than control. Endothion is significantly different from control only at the 5% level.

Some phytotoxic effects (leaf-spotting) were obvious on plots treated with Lebaycid, but plants were not seriously damaged.

TABLE 2. (Experiment 2.)—Materials and Dosages Used, Results, and Levels of Significance

Material	Dosage (Oz Active Ingred./Acre)	Mean Log (n + 1)	Equiv. Aphids (Mean Number per Plant)	Significance Levels* 5%	1%
Metasystox (i)	2 oz	1.598	3.96	a	A
Rogor	8 oz	2.007	10.16	a	B
PP 175	6 oz	2.209	16.18	b	BC
Lebaycid	12 oz.	2.342	21.98	bc	CD
Endothion	8 oz	2.435	27.23	c	DE
Control	Untreated	2.606	40.36	d	E
		s.d. = .056			

* Means designated by the same letter are not significantly different on Duncan's test.

Conclusions

The results of this trial indicate that a high degree of control (approx. 90%) can be obtained with a very low dosage of active ingredient of Metasystox (i). This result and that of previous experiments with this material as outlined by Lowe (1958b, 1960) show that the only occasion when this material gave better results than this against this insect was when 10 oz of the active material was used. On that occasion this dosage was credited with 100% control when applied from the air in 7 gal of water per acre. Materials giving less than 90% control are unlikely to achieve serious overall reduction of the pest in view of its acknowledged reproduction potential as temperatures rise and the early development of alates which can readily transfer to other crops.

Materials showing a control lower than this figure may, of course, be useful under other circumstances. It is also possible that the materials used in this experiment may give other results when used under differing circumstances. For instance, Rogor is generally acknowledged as having some contact, as well as a marked systemic effect. Similarly, Lebaycid is not usually recommended as having systemic properties. These materials could easily give a very different result when tried against this pest when it is present on open leaves during the summer or autumn

and more susceptible to contact effects. Inferences drawn from the results of this trial should therefore be confined to the limited circumstances of the trial, which represent a somewhat specialised case of the cabbage aphid as a pest.

Discussion

Under practical conditions of field application it should be noted that the materials used in this trial are subject to certain legal and health precautions under New Zealand conditions. Only those materials cleared for commercial use in New Zealand should be used on crops to be fed to animals or humans or applied from the air. All materials should be used with adequate operator attention to their respective hazards, as outlined by the New Zealand Department of Health and the Horticulture Division of the New Zealand Department of Agriculture.

Acknowledgments

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THE EFFECT OF CERTAIN CHEMICALS UPON SPROUTING OF POTATO TUBERS

II. The effect upon a crop grown from treated seed potatoes

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(Received 12 January 1961)

Summary

Seed potatoes were treated with two commercial dusts: one contained the methyl ester of alpha—naphthaleneacetic acid (MANA); the other contained tetrachlornitrobenzene (TCN). A study was made of the effect of these two chemicals upon: (a) the establishment of the seed potatoes; and (b) the subsequent crop.

Both dusts delayed establishment, MANA more than TCN, but the effect was reduced by exposing the treated seed to the air for three weeks before planting. MANA tended to reduce the number of plants finally becoming established, but TCN had no effect on final establishment. There was little effect upon total yield, but the proportion of table potatoes was increased with MANA and lowered with TCN. It is suggested that MANA increased apical sprout dominance, resulting in a smaller number of sprouts developing, with a consequent lower tuber set and higher average size of tuber. On the other hand, TCN may have decreased the dominance of the apical sprout, allowing more sprouts to develop and more tubers to set, with a consequent lower average size.

Introduction

In New Zealand a high proportion of the potato crop is not graded at harvest; the crop is stored as one grade and not separated into table and seed sizes until there is a demand for table potatoes. If the application of sprout inhibitors to potatoes to improve their storage life should become widespread (Driver 1957) it is inevitable that some seed tubers would be treated. North Island growers often have considerable difficulty in holding seed from one season to the next because warm conditions encourage rapid sprouting, and some method of sprout control would be desirable there. Consequently, it is necessary to know what effect the sprout inhibitors would have upon the subsequent growth of the seed.

The most important ways in which the inhibitors could have an effect would be in: (a) delaying emergence, (b) reducing final stand, (c) reducing plant vigour, (d) reducing yield, and (e) altering the proportion of table-size to seed-size tubers. It is important to know if the seed can be treated to overcome any deleterious effects of the sprout inhibitor, and the most practical way was considered to be the exposure of the tubers to the air for some weeks before planting to allow the volatile components of the inhibitors to evaporate.

Throughout the experiments commercial dusts were used, the one referred to as MANA containing 2.2% of the methyl ester of alpha-naphthaleneacetic acid, and the one referred to as TCN containing 3% of tetrachlornitrobenzene.

Experimental Methods and Results

1949–50a

Methods. Tubers of Aucklander Short Top, Chippewa, and Dakota from the 1949 storage trial (Driver 1957) which had been treated with the methyl ester of alpha-naphthaleneacetic acid (MANA) were taken straight from storage and planted in a replicated trial at Lincoln on 27 October 1949. The soil was warm and very dry. The untreated tubers established more quickly than the treated, but after six to eight weeks no difference could be observed in the foliage. Dry conditions prevailed throughout the season, and yields were light. The trial was harvested on 2 May 1950, and the produce graded into table, seed, and pig sizes.

Results. Yields are given in Fig. 1.

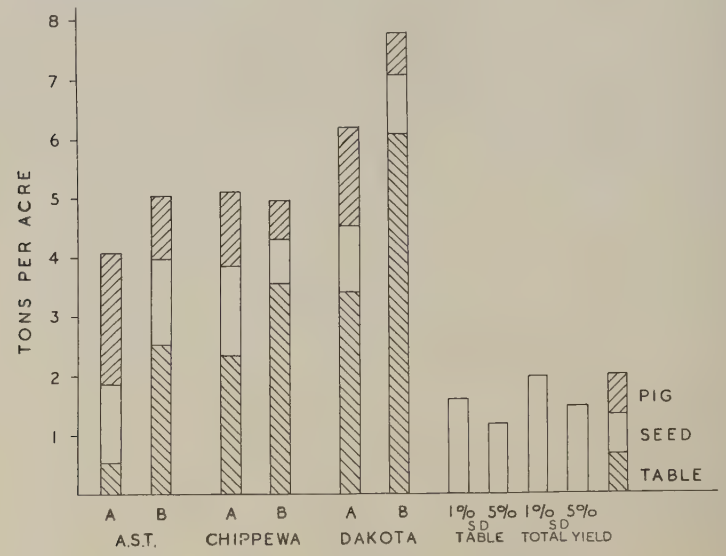

Fig. 1.—1949–50, Lincoln. A = Control. B = MANA-treated seed.

The yield of table potatoes was significantly higher from treated seed than from untreated. The treated seed gave a slightly later crop, which may have enabled the late variety to benefit more from rains later in the season; but the differences were as great with the earlier-maturing Aucklander Short Top as with the late Dakota Red, and in any case the difference in the yield of table sizes is not reflected in a proportionately higher total yield, but rather reflects a difference in the proportion of seed and pig potatoes to table potatoes.

1949–50b

Methods. A trial was laid down by the Department of Agriculture at Kerrytown, South Canterbury, to test the effect of tetrachlornitrobenzene (TCN) upon seed potatoes. Seed of Aucklander Short Top was treated with a commercial preparation of TCN in talc at the recommended rate of 10 lb per ton on 22 August 1949. The trial was planted on 4 October 1949 and harvested on 3 February 1950. TCN delayed the appearance of shoots for at least a fortnight.

Results. Results are given in Fig. 2.

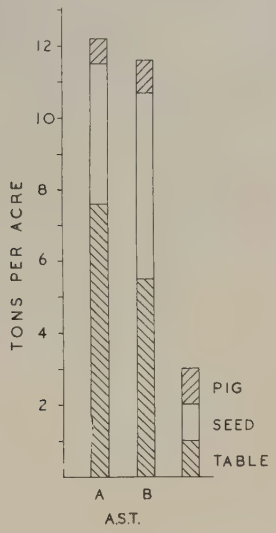

Fig. 2.—1949–50, Kerrytown. A = Control. B = TCN-treated seed.

In this trial a decrease occurred in the proportion of table potatoes in the crop grown from treated seed, and a significant increase in the yield of seed potatoes. There was no significant effect upon total yield.

A further small trial of paired plots comparing TCN at two different rates of application (4 oz and 10 oz per 140 lb bag) gave similar results; the yield of table potatoes from the lower application was between that of the control and the higher application.

1950–1

Methods. One hundred pound bags of uniformly graded tubers of Katahdin and Dakota Red were treated with commercial dusts containing MANA (at 3 lb per ton) and TCN (at 10 lb per ton) on 17 August 1950 and stored in a clamp. On 5 October samples of each treatment were taken from the clamp and placed in wooden sprouting trays in the light. On 26 October the remainder of each treatment was removed from storage and a trial planted using both the exposed and the unexposed seed material.

Exposure to the air almost entirely removed the inhibitory effect of TCN, so that establishment was nearly as rapid as with the exposed controls, and better than the controls planted straight from the clamp. The effect of MANA was partly removed by exposure to the air for three weeks, but early establishment was not as good as that of the controls. MANA-treated seed was erratic in emergence and did not catch up with other plots for at least six weeks. The trial was harvested on 2 May 1951, and the produce graded and weighed.

Results. The results are given in Fig. 3.

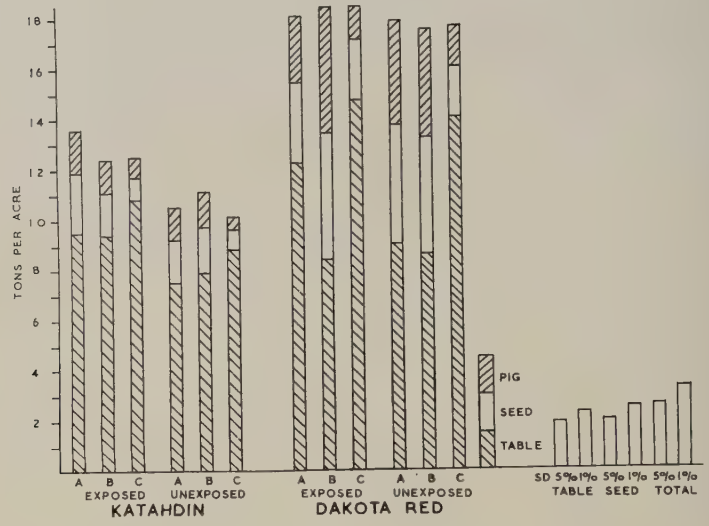

Fig. 3.—1950–1, Lincoln. A = Control. B = TCN-treated seed. C = MANA-treated seed.

Neither treatment had any marked nor consistent effect upon the total yield.

In every case there was a marked increase in the proportion of table tubers with MANA, and a corresponding reduction in the proportion of smaller sizes. The effect of TCN was less marked

but it tended to produce fewer table size tubers and more of seed size.

Exposure to the air for three weeks before planting assisted establishment of both treatments and the control. With the earlier variety, Katahdin, this resulted in a higher yield, both of table potatoes and total yield; but with the later variety, Dakota Red, growth following autumn rains partly obscured the effect of the earlier establishment.

1951–2

Methods. As part of a co-operative project with the Crop Experimentalist of the Department of Agriculture a trial was laid down at Lincoln using three varieties, Arran Chief, Aucklander Short Top, and Dakota Red. Seed had been treated on 15 August 1951 with either TCN or MANA, and an untreated portion was the control. The Lincoln trial was planted on 17 October 1951, and consisted of six replications of each variety-treatment combination, each plot being of four rows 25 ft long. The trial was harvested on 23 April 1952. A similar trial, under the control of the Department of Agriculture, Christchurch, was planted at Prebbleton on 25 October 1951, and harvested on 20 May 1952. This trial was laid down as a lattice-square layout with four replications.

The crop grew well in the early stages, but the summer was dry and autumn rains were late, so that the crop did not finish well. This resulted in a high proportion of small tubers.

Results. The results are given in Figs. 4, 5, and 6.

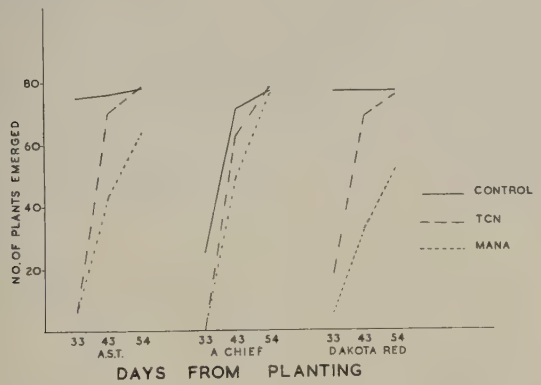

Fig. 4.—1951–2, Lincoln. Relative emergence of treated and untreated seed.

Both TCN and MANA delayed emergence, the effect being much greater with MANA. TCN had no effect upon the final stand, but with two varieties, Aucklander Short Top and Dakota Red, MANA reduced the number of plants establishing.

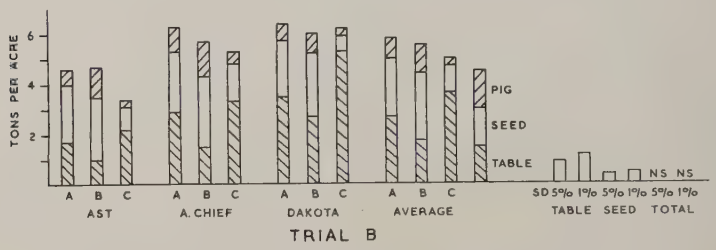

Fig. 5.—1951–2, Trial B, Lincoln. A = Control. B = TCN. C = MANA.

Fig. 6.—1951–2, Trial A, Prebbleton. A = Control. B = TCN. C = MANA.

In every case the MANA-treated plot gave the highest yield of table size potatoes, and the TCN-treated plot gave the lowest.

The MANA-treated plot in all cases has given the lowest yield of seed and pig sizes. The TCN-treated plot has given the highest yield of pig sizes, and in the Lincoln trial has given the highest yield of seed size tubers. In the Prebbleton trial the effect of TCN on seed size is obscured, as very few table size tubers were produced. Most tubers were graded as seed, and as TCN had a tendency to reduce total yield, this more than balanced the tendency to produce tubers of smaller than average size.

In general the control plot has given the highest total yield, and the MANA-treated plot the lowest.

Discussion

The results of all trials are consistent in showing that seed potatoes treated with MANA give a crop with tubers of above

average size, and TCN-treated seed potatoes give a crop with tubers of smaller than average size. The mechanism of the action of these chemicals is not known but some suggestions can be made. MANA reduced the final stand in the case of most varieties, giving a crop with plants spaced more widely than the controls. This alone could have had some effect upon the average size of tubers as it is well known that the proportion of large sizes is greatest at the widest spacings. However, the effect is just as great with the variety Arran Chief, with which there was only a slight reduction in final stand. Consequently, some other factor must be operating.

The yield per acre was similar for all three treatments. This implies that the number of tubers produced per acre was greatest after TCN-treatment, and smallest after MANA-treatment. As the number of tubers produced per acre is closely related to the number of shoots per acre, it is likely that the effect of MANA is to reduce the number of shoots, and the effect of TCN to increase the number of shoots. According to Michener (1942) the application of an auxin to sprouting potato tubers has the effect of increasing the apical dominance of sprouts. This would result in fewer sprouts developing per tuber, hence fewer stalks, fewer stolons, and fewer tubers set, giving the tubers the opportunity of growing to a greater size. It would appear that this is the mode of action of MANA. On the other hand, Michener (*loc. cit.*) found that a number of other chemicals which affected dormancy, such as ethylene chlorhydrin, had the effect of destroying auxin in the tuber, overcoming apical dominance, and allowing all sprouts to develop equally. This would result in more shoots developing, more stolons appearing, more tubers setting, and hence a smaller average size. TCN appears to have this effect upon seed tubers.

Acknowledgments

Thanks are due to Messrs Ivon Watkins Ltd. and Messrs Bayer Pharma Ltd. for the supply of chemicals, to the Department of Agriculture for permission to include their results, and to Mr H. W. Findon for technical assistance.

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SOIL DISINFECTION

XV. Effect of soil drenches on control of damping-off of pea seed

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(Received 25 October 1960)

Summary

The effect of dimethyl tetrahydrothiadiazine thione, sodium methyldithiocarbamate, and methylmercuric dicyandiamide on emergence of pea seeds was investigated in soil inoculated with *Pythium ultimum* Trow.

With increased inoculum potential the first two materials proved superior to the third in giving increased emergence and growth of seedlings.

Introduction

Chemicals for soil disinfection, other than those described earlier (Jacks 1956), are reaching the market. Three such materials, the active ingredient of which is distributed through the soil volume by using water as carrier, were tested for effectiveness in prevention of damping-off of pea seeds.

Materials and Methods

Preparation of Cultures

A 20% mixture of soaked shredded pea seeds in expanded perlite, moistened to 50% water-holding capacity, was sterilised for 20 min at 10 lb p.s.i., and inoculated with *Pythium ultimum* Trow. grown on potato dextrose agar. Cultures were incubated at 25°c for six days.

Soil Preparation

Sieved air-dried Manawatu sandy loam was inoculated with the cultures at 2% of soil weight. An amount of water, assessed by calculation (Jacks 1951), sufficient to raise soil moisture content to 25% water-holding capacity, was sprinkled over the soil and evenly distributed by sieving. Soil was placed in 6 in. pots. Inoculum for outdoor tests was prepared with soil in boxes in which two consecutive sowings of pea seeds were made to increase the inoculum potential of *Pythium*. Outdoor tests were carried out in plots 4 ft by 5 ft in area,

in which were drawn four equidistant seed drills, each of which received 1 lb of the inoculum.

Materials and Application

The following materials were used:

1. 85% dimethyl tetrahydrothiadiazine thione (Crag Mylone 85 W):
2. 31% sodium methyldithiocarbamate (Vapam); and
3. 6.3% methylmercuric dicyandiamide (Panogen).

Applications were made at concentrations given in Table 1, amounts required for the small areas of soil in pots and field plots being determined by calculation. In glass-house tests soil was saturated with 200 ml per pot, whilst 6.2 gal were required to saturate the soil in field plots to 10 in. depth. The amounts of water used corresponded to 31 gal per 100 sq. ft. Treatment 1, a granular material, was applied either by: (a) incorporating the granules mixed with 0.5 g of expanded perlite into the top 2 in. of soil in each pot, using a spatula, followed by 200 ml water; or (b) watering in of suspensions which were mechanically stirred until used. Checks received the equivalent amounts of water. Both in glass-house and field tests treatments were carried out seven days after soil inoculation.

Sowing and Records

In glass-house tests each pot was sown with 40 seeds of the variety Thomas Laxton (96% germination capacity) 10 days after treatment. Treatments were replicated six times. Pots were tray-watered. Three consecutive sowings were made to assess the effect of treatments on emergence in the presence of increased inoculum potential. Glass-house temperatures throughout the tests were 20c ± 8°c. Emergence records were made at two days' intervals, and completed 20 days after sowing. The velocity coefficient of emergence was assessed by the formula given earlier (Jacks 1958). In field plots, which were replicated five times, inoculated drills were each sown with 40 seeds 14 days after treatment. Three soil moisture tests were made, giving 27% ± 13% water-holding capacity at the time of sowing and during the six weeks' duration of the experiment. The total rainfall was 4.4 in., and the average temperature 17°c ± 1°c. Percentages of emergence were calculated on the basis of the germination capacity of seed. Plants were cut at ground level, dried at 80°c for 12 hours, and weighed.

Discussion of Results

In general, a decrease of emergence occurred with increased inoculum potential. Toxic residues of Treatment 1 depressed seed germination in the first sowing, these effects being more obvious with the granular form of application (Table 1). The therapeutic action of the lowest concentration of this treatment was of shorter duration

TABLE 1. RESULTS OF GLASS-HOUSE AND FIELD TESTS WITH PEA SEEDS SOWN IN *Pythium*-INOCULATED SOIL

Treatment	Concentration per 100 sq. ft.	Glass-house Tests									Field Test			
		Emergence			Equiv. Angle			Velocity Coefficient			% Emergence	Equiv. Angle	Velocity Coefficient	Weight Per Plant (g)
		Sowing			Sowing			Sowing						
		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd				
1	1 lb*	29	92	92	31	72	74	4.2	13.1	11.2	—	—	—	—
	0.5 lb*	82	82	56	65	66	51	4.4	15.6	9.9	—	—	—	—
	0.25 lb*	94	64	32	82	54	33	4.4	15.0	8.3	—	—	—	—
	1 lb	48	98	85	45	83	69	4.1	14.5	8.7	—	—	—	—
	0.5 lb	79	86	66	64	68	56	4.3	15.6	9.9	83.0	73.5	18.3	1.1
	0.25 lb	68	63	43	56	51	41	4.5	16.0	10.1	—	—	—	—
2	2 pts.	73	55	73	59	48	57	4.7	12.4	5.5	—	—	—	—
	1 pt.	65	53	67	54	47	55	3.9	11.1	3.6	70.0	58.7	16.3	1.2
3	0.76 pt.	70	56	1	57	48	3	5.3	10.3	7.5	20.4	26.9	11.1	0.2
	0.38 pt.	63	46	0	53	43	0	5.6	8.9	0	—	—	—	—
Check	—	53	6	3	46	11	6	5.3	11.1	7.6	9.6	12.5	10.0	0.2
Significance at 1%					7	10	16	—	—	—	—	21.9	—	—

* Granules mixed with surface soil and washed in.

than the higher concentrations, but allowed earlier sowing after treatment without injury to germination. The higher concentrations of Treatment 1 were, in general, more effective than all other treatments. Treatment 2 was almost as effective as Treatment 1, and significantly better than Treatment 3 after the third sowing. Treatment 3 gave shorter protection from damping-off than Treatments 1 and 2, but brought about significantly better emergence over checks in the first two sowings. The effects of speed of germination versus growth of *Pythium* were not obvious under the conditions of these experiments. However, compared with checks, the speed of emergence was increased by Treatment 1, with the exception of the first sowing where toxic residues depressed and delayed emergence. Treatment 2 delayed emergence in the third sowing. In the field test, Treatments 1 and 2 gave significantly higher emergence and better plant growth than Treatments 3 and check.

Conclusion

A delay of two to three weeks between treatment and planting would be necessary for materials 1 and 2, whilst 3 did not injure existing plants or reduce germination in tests not reported here. The effective period for control of damping-off was longest for Treatments 1 and 2, pointing to their use for intensively cultivated garden and nursery soils, with high inoculation potential. Treatment 3, however, may be used effectively in borders, where its application will not injure plants and reduce damping-off of seeds and seedlings. Distribution of Treatment 1 in granular form opens possibilities for convenient application in the field, provided the depth of incorporation and the extent of dispersion through the soil are satisfactory.

Acknowledgments

Grateful acknowledgment is made to Ivon Watkins, Ltd., New Plymouth for a special grant in support of investigation of chemical control methods of soil-borne diseases and pests.

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METHODS OF MEASURING GROWTH IN SHORT TUSSOCKS

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(Received 22 November 1960)

Summary

Four methods of measuring growth rate of short tussocks were compared. These were:—

1. Rates of elongation of younger leaves in a tiller.
2. Micro-measurement of basal perimeter.
3. Total yield of plants.
4. Yield from clipping.

The measurements of leaf elongation and of basal perimeter appeared to have most promise as field techniques.

Introduction

Prior to the study of the effect of any treatment on a plant it is necessary to have a method for measuring the plant's response, e.g., growth, and to know the characteristics of the method being used.

This paper describes some possible methods for measuring the growth rate in short time intervals of native short tussocks: fescue tussock (*Festuca novae-zealandiae* L.), silver tussock (*Poa caespitosa* L.), and blue tussock (*Poa colensoi* L.). Four general methods were tried: (a) those based on rates of elongation of younger leaves in a tiller, (b) micro-measurement of rate of increase of basal perimeter, (c) total yield of whole plants, and (d) yield from clipping to particular heights.

All investigations were carried out using clonal material kept in controlled climate cabinets to minimise genotypic and environmental variability.

Methods

Rate of Leaf Elongation

Preliminary work showed that most of the growth of the tussock was confined to the youngest leaf in a tiller, and only in $\frac{1}{4}$–$\frac{1}{2}$

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of the cases was the second youngest leaf increasing in length. Because of this situation it was found that the coeffcient of variability between tillers was lower if the elongation of the youngest visible leaf only was considered rather that total leaf elongation by a tiller. There is a diurnal fluctuation in rate of leaf elongation, the early morning rate being about half the mid-afternoon rate. Hence it is desirable to standardise measuring time.

The method finally adopted was to mark the youngest leaf in a tiller with a numbered jeweller's tag and to measure the distance from the tip of the leaf to a metal ring in the ground at the base of the tussock. This could be measured with an accuracy of ± 1 mm. The label was changed to a new leaf if the existing leaf ceased to grow (i.e., grew less than 1 mm/day), or if it exceeded 30 cm in length (20–25 cm in blue tussock). The leaves were measured at 3-day intervals. One man was able to measure 70-90 leaves per hour.

Even in a constant environment the growth rate of a plant may vary systematically with time. Over a 2–3 week period this may amount to only 2–3%, but for determinations made with the same plant in the same environment several months later the values may differ by 15%. Hence the growth rate is not completely independent of the pre-treatment received. Although there are differences in rates of growth between plants of the same clone, the fractional change of growth rate accompanying a treatment change is approximately constant for all plants of a clone (to within ± 3–4%) (Scott 1961).

The number of leaves to be measured will depend upon the accuracy required. Table 1 gives for leaves of fescue and blue tussock the standard error of the mean increment of groups of five leaves expressed as a percentage of the mean, for different number of sampling dates (d) and number of five leaf samples per date (n).

TABLE 1. Relation of S.E. to Leaves Sampled and Sampling Date

n \ d	2	3	4	6	10
1	22	18	15	12	10
2	15	12	11	9	7
3	12	10	9	7	5
4	11	9	8	6	5
6	9	7	6	5	4
10	7	5	5	4	3

The value for silver tussock is about ¾ of that given for fescue and blue tussock in the table. Since the growth varies with time it is advisable that for the degree of accuracy required the number

of sampling dates should be made large at the expense of samples per date.

Under optimum conditions the average growth rate of the youngest leaves in the three species is about the same, and is in the range .7–1.5 cm per day.

Micro-measurement of Basal Perimeter

This was measured using a micrometer and an expanding ring around the base of the tussock on which there were two reference measuring points. The expanding ring was a $\frac{3}{4}$ in. long slotted metal tube attached by an adjustable clamp to a $\frac{1}{2}$ in.-wide plastic strip which passed around the base of the tussock and which was fastened to a $\frac{3}{4}$ in. length of rod fitting into the tube. The metal portion formed the expanding part of the ring and had two protruding lugs for reference points. The micrometer used was accurate to 0.001 in. and was fitted with a ratchet to give measurement at even pressure. The reading on the micrometer continued to show a decrease after the ratchet had started to "free wheel", though gradually reaching a steady value, and it became standard practice to take 50 half turns on the ratchet after it had commenced to free wheel. With this procedure it was possible with four successive determinations to get the mean value of the perimeter with a standard error of $\pm$ 2–4 $\times$ 10^{-3} in.

A trial was conducted using a silver tussock plant with a basal perimeter of about 8 in. The time/perimeter length graph was approximately linear and the gradient varied with the treatment. There was an increase of 0.008 in. per day at 15°c and 0.013 in. per day at 5°c. As the magnitude of these figures indicate, daily differences could be detected.

If a plant wilted or was subject to considerable moisture stress the reading for the basal diameter could decrease by 0.100 in. or more.

Total Yield of Whole Plants

For clones this method requires the subdivision of a large plant to give many small plants which may then be harvested at successive stages in an experimental programme. It was found that 1–2 tiller plant cuttings could be established. The best medium found comprised a fine pumice and peat mixture watered daily with nutrient solution.

A trial with silver tussock plants that had reached the 5–10 tiller stage showed that after 23 days at a mean temperature of 5°c plants had made an average gain of .54 g, while at 15°c the average gain was 1.46 g. There was a standard deviation of about 40% of the mean value. The ratio of .37 between the two treatments was almost identical to that obtained by the leaf elongation method.

Yield from Clipping

The method tried was to cut all leaves to a constant height (about 7¼ in. above ground surface) and then at subsequent three-day periods recut and collect leaf material that had grown above the cut surface.

The method was not a success owing to a period of rapid stimulation of growth following cutting which was succeeded by a marked decline. For example, with plants of silver tussock the daily increase above the cut surface was 0.26 g/day immediately following the cutting, but fell to 0.12g/day after two weeks. Also there is a large error in the measurements, due to the small increment in length of leaves appearing above the cut surface and the difficulty of accurately cutting back to specified surface. Because of this and the initial stimulating effect of cutting, the responses of the plants to experimental treatments are masked.

Comparison of Methods

The dry weight gain of the whole plant gives the most overall measurement of growth; it is simple and it has reasonable accuracy (coeff. of variation 40–50%). Its disadvantages are that it requires at least a two-three week treatment period, is destructive in nature, is dependent upon the plant size, and is probably not applicable to field investigation.

Measurement of rate of leaf elongation is relatively accurate, can give suitable results in three days or less, is non-destructive, is applicable to any size of plant, and could probably be used in the field. Its principal disadvantage is that it is time-consuming. The single comparison indicated that it showed proportional changes in amount of growth almost identical to those obtained by the total yield method.

The advantages of the basal perimeter method are its simplicity in measurement, accuracy, and applicability to field as well as laboratory studies. Its disadvantages are its dependence on the size of the plant and the turgidity of its tissue. The latter may be an indirect advantage for studies of moisture stress. Results obtained by this method are not comparable to those obtained by the other methods, e.g., basal perimeter increased by about 50% more at 5°c as compared with 15°c, whereas growth assessed by the two previous methods was about 60% less at 42°F than at 60°F. Hence in intensive work more than one method may have to be used to measure the change in pattern of growth.

The clipping method shows little promise, though the clipped leaves may be used as "marker" leaves in the rate elongation method as described by K. F. O'Connor (pers. comm.).

Acknowledgments

The author wishes to thank Mr A. C. Glenday for assistance with the statistical work.

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HOLLOW HEART OF PEA SEED
(A Note)

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(Received 5 April 1961)

Summary

An abnormal condition of pea seed characterised by the presence of sunken areas in the cotyledons was shown to be distinct from Marsh Spot, a deficiency disease which causes somewhat similar symptoms. The cause of the Hollow Heart condition was not determined but it was shown to develop during the drying of the seed.

Introduction

Myers (1948) described an abnormal condition of garden pea seed which she called Hollow Heart. The editor of the journal in which her paper appeared added a footnote to the effect that this condition was the same as that previously described as Marsh Spot, which had been shown to be caused by a soil deficiency of manganese (Löhnis 1936; Pethybridge 1936; De Bruyn 1939). The condition has again been found in commercial pea seed, and recent work in New Zealand has shown that Hollow Heart is quite distinct in etiology from Marsh Spot. In the United States "cavitation" has been described (G. E. Nutile pers. comm.), and co-operative investigations with workers in other countries indicate that "cavitation" and Hollow Heart are identical (M. Noble pers. comm.).

The symptoms of Marsh Spot were first described by De Bruyn (1933) who stated that the flat inner surface of one or both cotyledons, while still enclosed in the seed coat, showed a brown lesion of varying extent, sometimes accompanied by partial or entire necrosis of the plumule. Subsequent workers have confirmed that the primary effect of manganese deficiency is to produce a brown lesion on the cotyledons. Hollow Heart, however, was described by Myers (1948) as cracked or sunken areas in the centres of the cotyledons, occasionally accompanied by discoloration and apparent necrosis.

Experimental Methods and Results

In the course of the present investigations several thousand seeds of all the commonly grown New Zealand garden pea varieties were examined for the presence of Hollow Heart. Discoloration of the hollow was found only when the seed was germinated in soil or sand. No discoloration or necrosis was ever found when cotyledons were split apart following a 12 hour soak in tap water. (Fig. 1).

Fig. 1.—Inner face of pea cotyledons showing typical symptoms of Hollow Heart.

A more fundamental difference between the two conditions has also been noted. Marsh Spot is a deficiency disease which develops in the growing plant. De Bruyn (1933) noted that the percentage of affected seeds increased as the seed approached maturity, while Lacey (1934) described it as present on both green and dried pea seed. Hollow Heart, however, was described by Myers (1948) from dried seed, and has never been seen in fresh peas.

TABLE 1. Hollow Heart in Fresh and Dried Pea Seed

Variety	Percentage Hollow Heart	
	Fresh	Dried
Onward	0	10.0
Onward	0	19.6
Wm. Massey	0	18.4

In an attempt to find it in fresh peas, samples of 500 seeds each were taken from three mature crops in the Lincoln area: two of the variety Onward, and one of the variety William Massey (Kelvedon Wonder). Half the seeds in each sample were opened immediately and examined for Hollow Heart, while the remainder were dried and then soaked in tap water before being opened. The results are shown in Tabe 1.

Conclusions

On the basis of symptoms alone, Hollow Heart differs from Marsh Spot in that the hollows are not normally necrotic and the plumule is undamaged. Moreover, the results shown in Table 1 indicate that the Hollow Heart condition develops during drying of the seed and not during the growth of the crop. It is therefore quite distinct from Marsh Spot, and indeed is unlikely to be directly caused by soil conditions of any kind.

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THE ARGENTINE STEM WEEVIL *Hyperodes bonariensis* Kuschel ON PASTURE IN AUCKLAND

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(Received 22 March 1961)

Summary

In the Mangere district of Auckland long-term pastures were found to support heavy infestations of the Argentine Stem Weevil, *Hyperodes bonariensis* Kuschel. Established pastures were less damaged than young or newly sown pastures. White clover tended to invade areas denuded by the weevil, thereby producing an unbalanced sward. Paspalum and 30–40 year old pastures were virtually uninfested.

The duration of the breeding season in Auckland in 1959-60 was somewhat similar to that in the South Island in 1955-7: the Auckland season began in mid-July (approximately six weeks earlier than in the South Island), and ended in mid-April (approximately four weeks earlier).

Late April is suggested as the most suitable period in which to apply control measures on existing pasture. Newly sown areas may possibly be protected by the application of an insecticide soon after the seedlings appear, provided adjoining paddocks have been treated.

Introduction

The results of a survey of the weevil on ryegrass in Canterbury was published by Kelsey (1958). In 1960 Hoy published a preliminary assessment of the extent and severity of damage to ryegrass in the North Island, where he considered the most serious attacks to be in Taranaki, with only light damage in Auckland province.

The present survey was conducted for one year in the Mangere district of Auckland. Particular attention was given to noting possible differences, in the life history and behaviour of the weevils, from the characteristics described by Kelsey (*loc. cit.*) for the South Island.

Experimental Methods and Results

Methods

In the main part of the survey four 4 in. sq. turf samples were taken, at weekly intervals, from the same paddock (referred to as Barn Paddock), over a period of 12 months, with the exception of May and June, when fewer samples were required.

Each sample was dissected in the laboratory, on a sheet of white paper on which moving adults could easily be seen. Each grass stem was examined individually for eggs and larvae, after which all soil and debris were placed in troughs, and flooded. Pre-pupae, pupae, and adults came to the surface, usually within 10 minutes.

Complementary information was gained by sweep-sampling of adult weevils, at grass level, carried out after dark, in approximately similar weather conditions. A sample consisting of 50 paced sweeps with a 12 in. diameter net was taken in two sectors of a paddock. Results from the two methods were found to be well correlated ($r = 0.68$, $P = 0.046$), the latter giving a good estimate of the rise and decline of a population.

Other Phytophagous Insects

Adults of another weevil, *Mandalotus miricollis* Broun, were very commonly found, both in turf samples and by sweeping. A note, written by Mr T. G. Campbell, of the Division of Entomology C.S.I.R.O., Canberra, Australia, attached to a specimen of a similar weevil in the collection of Plant Diseases Division, D.S.I.R., states: "Examined by H. Womersley who considers that it appears to be identical with *M. medcoxalis* Lea". It seems probable, therefore, that this weevil is an introduction to New Zealand from Australia, although the synonymy has not been published. The adults fed on the same grasses as *Hyperodes*, but chewed from the sides of the leaves towards the midrib, instead of from the leaf surface. No larvae were found, nor any damage to grass which could be attributable to them. Pupae were uncovered about an inch below soil level, on one occasion only.

The elaterid beetle *Lacon van de Polli* Cand. was extremely common and its larvae plentiful in the soil at all times.

The agromyzid fly *Cerodontha denticornis* Panz. and the ephydrid fly *Hydrellia tritici* Coq. were abundant. The larvae of both species are leaf miners. Those of *C. denticornis*, a species which has long been recognised as a pest of Gramineae, were present during most of the year. *H. tritici*, suspected by Cumber and Harrison (1959) of being damaging, was present in sufficient numbers to constitute a pest during the spring (May 1960).

Host Plants

Adults of *Hyperodes* fed in the laboratory on all pasture plants offered. Annual meadow grass (*Poa annua* L.), rough meadow grass (*Poa trivialis* L.), ryegrass (*Lolium* sp.), and cocksfoot (*Dactylis glomerata* L.) were preferred. Brome (*Bromus* sp.), Yorkshire fog (*Holcus lanatus* L.), paspalum (*Paspalum dilatatum* Poir.), and white clover (*Trifolium repens* L.) were also eaten, and were chewed extensively when no alternative was given.

Eggs were laid freely, and larvae became established only in the meadow grasses, ryegrass, and cocksfoot. Eggs were seen on only one

occasion in Yorkshire fog, and two eggs were also noted in the midrib on the underside of a young cat's ear (*Crepis* sp.) leaf. Paspalum was conspicuously free from attack. No eggs or larvae were discovered on either white or red clover. Cocksfoot was damaged by *Hyperodes* larval feeding to a much lesser extent than ryegrass.

Life History

H. bonariensis passed the winter in the adult stage. Adults in captivity were seen *in copulo* during May, June, and July, and the first eggs in the field were discovered on *Poa annua* on 12 July, 50 days earlier than in the South Island.

Larvae were present in samples from 7 September (S. Island—last week in September), and pupae from 2 November (S. Island—last week in November). The adult population began to increase in mid-November (S. Island—beginning of December). Second generation eggs were present from 25 November (S. Island—17 December), reaching their peak at the end of December.

The numbers of adults, eggs, and larvae collected in the period late January to early March suggested a third generation, although only the peak for larvae is truly significant. However, since it is unlikely that adults would persist during this favourable seven weeks period without ovipositing, it is probable that a partial third generation occurred. Larvae and pupae were found until mid-April (S. Island—mid-May).

Kelsey (*loc. cit.*) showed in his survey that eggs were absent during five weeks in November–December, and larvae during four weeks in December–January. In Auckland both eggs and larvae were present at all times from their initial appearance in spring until the end of the season.

Adult weevils were present throughout the year. Their numbers rose to a peak at the end of February, declined to approximately 7% of the peak level during the winter months, and dropped still further during October prior to the emergence of a new generation in November.

The adult life span extended over several months. Newly emerged adults, fed on ryegrass in the laboratory, lived for periods varying from 62 to 179 days. One weevil kept without food lived for 27 days.

Breeding experiments in the insectary showed the life cycle to be considerably more protracted in the early part of the season than during the summer months. (Table 1). Larvae were extraordinarily difficult to observe accurately over a long period, owing to their habit of migrating from one grass stem to another. The duration given for the larval stage must therefore be regarded as only approximate. The "pre-pupal stage" was taken to occur between emergence of the fully fed larva from the grass stem and its pupation in the upper soil. The "pupal stage" included the pharate adult up to the time of its ecdysis from the pupal cuticle (Hinton 1958).

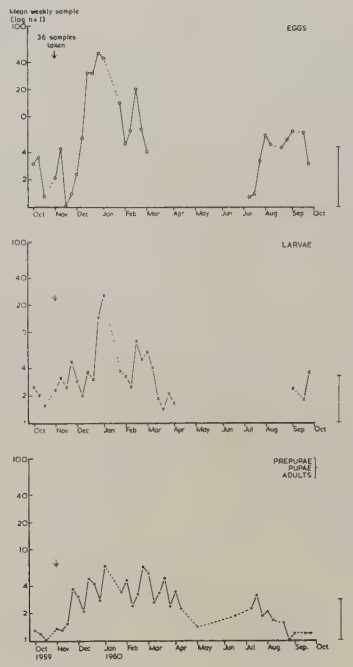

Fig. 1.—Life cycle of *H. bonariensis* as shown by the mean number of insects from four samples weekly from Barn Paddock. Confidence bands (95%) are given for difference between two weekly means. Dotted lines represent samples more than one week apart.

TABLE 1. DURATION OF STAGES IN THE LIFE CYCLE OF *H. bonariensis* IN AUCKLAND

Stage	Month	Duration (No. of Days)
Egg	July/August	33–34
	November	11–12
	December	9–10
Larval	August/October	50–66
	December	21–24
	January/February	14–21
Pre-pupal	November/December	6–12
	February	4
Pupal	November	11–13
	December	10–12
	January/February	7–8

Habits

Adults. Adults are nocturnal feeders, a habit which was of great assistance in population sampling. The weevils tended to crawl to the tips of leaves to feed, soon after dusk, and were thus in a good position to fall into the sweeping net. In the process of chewing, leaf tissue was scraped away between the veins, which were left torn and curling. The upper (abaxillary) surface was generally preferred.

Very little evidence of flight was recorded. Grease traps, of the type used for aphid sampling (Close 1959), were in position throughout the spring and summer, but caught only four individuals. No mating or dispersal flights were seen. Pairs mated freely in captivity.

Females were observed to lay up to 37 eggs over periods of up to 40 days. Copulation took place at intervals throughout the period of egg laying. Eggs were laid initially in groups of two or three, although one group of five was recorded, the eggs being laid end to end. Later in the sequence the eggs tended to be laid singly or in pairs.

During the peak period in early January, eggs, usually inserted in the leaf sheath within 2 in. of ground level, were often placed haphazardly in other positions. Not infrequently, pale green eggs were noted, intermingled with the normal greenish-black ones.

Grasses preferred for oviposition varied according to the season. Annual meadow grass germinated in late June, and eggs were present in the more robust seedlings on 12 July. This grass, together with rough meadow grass, carried a large larval population up to mid November. Eggs were first noticed on ryegrass on 10 August and in cocksfoot two weeks later. In general, ryegrass carried a larger population of *Hyperodes* than cocksfoot except under very dry growing conditions when ryegrass became less succulent.

Larvae. Larvae were found to pass through seven instars. This was determined, according to Dyar's Law, by measurement of the width of larval head capsules (Table 2).

TABLE 2. Measurements in mm. of Larval Head Capsules

Instar	No. of Individuals	Mean	Standard Deviation	99% Range
1st	64	0.194	0.009	.170–.218
2nd	30	0.258	0.011	.228–.288
3rd	34	0.300	0.009	.278–.322
4th	44	0.357	0.009	.334–.380
5th	28	0.414	0.013	.381–.447
6th	48	0.487	0.010	.461–.513
7th (pre-pupal)	21	0.539	0.011	.511–.567

Larval behaviour in ryegrass was much as described by Kelsey (*loc. cit.*). It was noted, however, that larvae did not necessarily

remain in the stem that they first entered from the egg, small stems being abandoned when space for feeding was exhausted.

The presence of a weevil infestation was first indicated in early spring by yellowing of the young leaves on tillers of meadow grass. In the prevailing humid conditions, these remained erect for several days after their supply of sap had been cut by larvae in the lower part of the stem.

Damage produced by *Hyperodes* larvae, even in dead tillers, was easily distinguished from that caused by fly larvae, by the type of excreta left in the stem. Weevil excreta was in the form of fibrous frass, while fly larvae evacuated a dark slimy substance, which dried to black specks between the surfaces of leaf sheaths.

In cocksfoot larvae did not penetrate to the centre of the stem but tunnelled instead in the fleshy leaf sheaths. The tiller died only if it was eaten at the base, as sometimes occurred when larvae were approaching the pre-pupal stage.

Pre-pupae and Pupae. When larvae were fully fed, at approximately 6 mm length, they entered the pre-pupal stage, then migrated from stem to soil, burrowing extensively in the top ¼ in. before preparing a cell in which to pupate.

A smooth-walled, ovoid to spherical cell was formed by rotary movements of the body. In this the pre-pupa lay for several days, in a curled position, before pupating.

In this stage the weevil thrived best in slightly damp soil, such as might be associated with heavy dew. In exposed, dry, powdery soil, or in waterlogged soil, most pre-pupae died.

Pupae, observed in a glass dish, were active up to shortly before ecdysis, which occurred inside the cell. The newly emerged adult broke through the cell wall while still in a teneral condition. The normal dull grey colour was assumed within two to three days.

Survey of Barn Paddock

An intensive survey of Barn Paddock was undertaken on 3 November 1959. Barn Paddock had been sown three years previously with a ryegrass–clover–cocksfoot mixture. A year later 2/3 of the paddock was resown owing to its poor condition.

Thirty-six turf samples, each 4 in. square, were taken from approximately equidistant (paced) stations. Thus 12 were in the three-year-old section, and 24 were in the two-year-old section, of the paddock.

The results (Table 3) showed twice as many eggs, larvae, and pupae in the older, as in the younger, area. Since only one adult was found, these would all have been produced by the overwintering generation. This could be explained by a similar rate of population growth in the two areas, with a doubling in numbers each season, the population in the newer area being one year

TABLE 3. Comparative Populations of *H. bonariensis* and Related Coverage per Sq. Ft. on 2-Yr.-Old and 3-Yr.-Old Areas of Barn Paddock on 3 November 1959

Stages	3-Yr-Old Area	2-Yr-Old Area
Eggs per sq. ft.	24.75	12.38
Larvae per sq. ft.	36.00	12.38
Pupae } per sq. ft.	2.25	7.50
Pre-pupae }		
Adults per sq. ft.	.00	.38
Grass plants per sq. ft.	82.50	110.00

younger. Furthermore, the grass coverage was found to average 82.5 plants per sq. ft. in the older area, as against 110 plants per sq. ft. in the two-year-old portion. A difference as large as this would only happen by chance once in 100 times, if the two areas were the same. Assuming plant growth to have been similar after each sowing, the results indicate that the heavier weevil population had considerably reduced the grass coverage.

Clover Domination in Pasture

Later in the season, in Barn Paddock, it was noticed that white clover had invaded all ground not covered by grass or weeds. This unbalanced condition of the pasture is one which, in certain circumstances, can give rise to bloat in cattle. (Gilder 1942; Johns 1959). It is thus possible that *Hyperodes* could contribute to the incidence of this disorder.

A nearby pasture, sown in autumn 1959 with a standard ryegrass–clover–cocksfoot mixture, had become so predominantly covered with white clover by spring 1960 that cattle were being grazed on it for only a few hours at a time. The infestation of *Hyperodes* on the remaining patches of grass greatly exceeded that in Barn Paddock.

Susceptibility of Various Types of Pasture

Evening sweep sampling, combined, in some cases, with turf sampling, gave an indication of the density of weevil population carried by differing types of pasture. The samples were taken in similar weather conditions, as far as possible, and between mid-February and mid-March, when the number of second generation adults was at its peak. Grass length was moderate in most cases, but where it was short the catch of adult weevils was light, due to the mechanics of sweeping. Barn Paddock, a standard pasture carrying a weevil population under regular observation, was used for comparison.

It was apparent from these samplings that paspalum was largely immune from weevil attack, and that very old mixed pasture (30–40 years old) had developed a coverage of grasses and weeds which was similarly resistant. A predominantly cocksfoot pasture carried a heavy

population. A moderate catch was obtained from a mixed pasture used as an airfield, and kept short. Newly sown pastures were found to be particularly vulnerable. This was also noted by Hoy (*loc. cit.*) for the North Island in general. The position was undoubtedly aggravated when neighbouring paddocks were heavily infested.

Once pastures were established, the damage done by *Hyperodes* was largely offset by the rate of plant growth, except during periods of drought. At such times, and after hay harvest, a heavily infested pasture was noticeably slow to recover.

Both Kelsey (*loc. cit.*) and Hoy (*loc. cit.*) found that short-rotation and Italian ryegrass strains suffered more serious damage than perennial ryegrass. Long-term pastures, which typically contain 25 parts perennial ryegrass plus five parts cocksfoot to four parts Italian or H1 ryegrass, are usual around Auckland. This fact, coupled with good growing conditions, allows pastures in this part of New Zealand to harbour relatively large *Hyperodes* populations without undergoing such obvious destruction as occurs in the South Island.

Control

Kelsey (*loc. cit.*) stated that adult weevils may be controlled with both DDT and lindane, and that these would have to be used against each of the two generations. No tests of the efficacy of specific insecticides were carried out in Auckland, and discussion in the present paper is confined to the most effective periods for their application.

The three generations probably existing in Auckland overlap to such an extent as to render treatments of doubtful value. Eggs and larvae, being enclosed within the grass stems, are virtually immune to those insecticides which are considered safe to use on pastures. Control measures must therefore be directed against the adult weevils, at the time when they alone are present, that is, between mid-April and early July.

For existing pasture late April would appear to be the most suitable period; June and July are usually too wet: insecticides are leached away too quickly, and paddocks become inaccessible. The application of a therapeutant with top dressing in spring is unlikely to be successful, since eggs are present in large numbers by August. This conclusion is supported by observations made on a moderately infested pasture top-dressed with DDT superphosphate on 31 August. The number of second-generation eggs present on 20 December was less than would be expected if no control had been applied, but nevertheless sufficient remained for a considerable build up of weevil population later.

To protect new pasture, it would be important to treat adjoining paddocks prior to sowing, and to apply an insecticide on the sown area itself soon after the seedlings appear.

In view of the difficulty of applying safe insecticides effectively, the discovery of an egg or larval parasite would appear to offer the best hope for control of *Hyperodes*. No natural enemies were recorded during the present survey.

Acknowledgments

Acknowledgment and thanks are extended to Dr H. R. Thompson and Miss Marilyn Ward, of the Applied Mathematics Laboratory, D.S.I.R., for their assistance in the preparation of graphs and statistics; to Mr Peter Pottinger, of Lincoln College, Christchurch, for information on his population sampling methods; to Mr Clyde Ellett, of Ihumatao, Mangere, for the use of Barn Paddock; and to Mr Ellett and other farmers in the district for their cheerfully given assistance.

We are indebted to the Entomology Division Sub-Station, D.S.I.R., Lincoln, for information on the identity of this weevil, formerly known as *H. griseus*. Specimens have been recently checked by Prof. G. Kuschel, the acknowledged authority on South American weevils, who stated that all New Zealand material had hitherto been misidentified, the correct name being *Hyperodes bonariensis* Kuschel (Kuschel 1955).

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A PROTEOLYTIC RUMEN ANAEROBE

(A note)

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Summary

Clostridium bifermentans was isolated from the bovine rumen, and may be of importance in rumen proteolysis.

Introduction

The breakdown of protein in the rumen and its incorporation into microbial protein has been shown by McDonald (1954, 1957) and many others. Numerous attempts have been made to isolate proteolytic rumen bacteria, but most of the organisms obtained have been facultative anaerobes, often isolated with an alternative carbon source in the medium. Thus Appleby (1955) isolated *Bacillus licheniformis* and other organisms from sheep, and Hunt and Moore (1958) isolated a proteolytic *Flavobacterium* from a cow. More recently Blackburn and Hobson (1960) isolated several facultative anaerobes, which they suggested represented only a fraction of the proteolytic organisms of the rumen. An unidentified anaerobic bacterium (RO–Cl) with proteolytic activity was isolated by Huhtanen and Gall (1953).

Experimental

In the course of a search for rumen bacteria capable of attacking *Streptococcus bovis dextran* (Clarke 1959) an anaerobic, proteolytic, gram positive, sporing bacterium was isolated from the rumens of four cows. The organism was present in numbers ranging from 10^4 to 10^8 organisms/ml of rumen liquor. A further isolation was made on the same dextran peptone medium 12 months after the first isolations. Spores of the organism could not be demonstrated in rumen liquor, and no isolations could be made after heating rumen liquor at 80°c for 20 min.

The organism was identified as a non-toxic strain of *Clostridium bifermentans*. It was actively proteolytic, digesting and blackening iron milk and cooked ox heart, and rapidly liquefying gelatin. Acetic

acid was the main product of glucose. Formic and lactic acids, traces of propionic, butyric and succinic acids, and a small amount of gas were also formed.

As the organism was present in the rumen in reasonably high numbers, and as it was isolated on several occasions, it is probably a normal inhabitant of the rumens of New Zealand cows, and is probably of importance in rumen proteolysis. Although *Clostridium bifermentans* has a wide distribution in soil (Breed *et al.* 1957), its concentration in the vegetative state in the rumen precludes its being merely a rumen passenger.

Acknowledgments

The author is indebted to Prof. L. S. McClung of the Department of Bacteriology, Indiana University for demonstrating indole production by the organism, and for confirming its identification.

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THE DISTRIBUTION OF MOLYBDENUM IN THE SOILS OF NEW ZEALAND

II. Soils of the South Island

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Summary

The range of acid oxalate-soluble molybdenum levels found in soils of the South Island is shown by distribution tables.

There is much less variation among these soils than was found in the North Island soils previously discussed.

The main points of difference between soil groups are:

(1) Yellow-grey earths with damp climate and soils intermediate between yellow-grey and yellow-brown earths of the downs and hills have lower molybdenum levels than any other group. These soils respond markedly to molybdate top-dressing.

(2) Soils formed on greywacke, tertiary rocks, and sediments derived from these in Marlborough have slightly higher levels than members of the same soil group in Canterbury and Otago.

(3) Soils derived from Moutere gravels and the podzols of Westland are extremely low in molybdenum.

INTRODUCTION

Part 1 of this series (Grigg 1960) dealt with soils of the North Island. It was shown that both the total molybdenum and the fraction soluble in acid ammonium oxalate solution varied according to the parent material and the degree of delevopment of the different soil types. In this second paper South Island soils will be similarly considered.

The majority of South Island soils are derived from parent materials such as greywacke, alluvial or aeolian products derived from greywacke, granites, and mica schists. All of these have rather similar soil-forming properties, giving rise to zonal soils (Taylor 1948). There is little complication from volcanic materials, although these do occur in certain localities.

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The predominant original vegetation in the South Island was tussock grassland on the east coast and rain forest in the west and south. Consequently the concept of the "suite" or leaching sequence of soil types derived from a common parent material under conditions of changing vegetation is less applicable than in the North Island. Temperature and rainfall are therefore the main factors which might be expected to affect the molybdenum status of these soils.

No complete soil survey of the South Island on the 4 mile-to-1 inch scale as in the North Island General Survey (N.Z. Soil Bureau 1954) has yet been published. Several district surveys (e.g., Gibbs 1950; Gibbs and Beggs 1953; Wright *et al.* 1952) have been made, however, and additional unpublished data have been made available to the Department of Agriculture by Soil Bureau officers. A modification of Taylor's (1948) classification has been used as the basis of the groupings in this paper, soils being arranged according to parent material.

Kidson (1954) has discussed the molybdenum content of soils of the Nelson province, and Wells (1956) considers South Island soils in his discussion of molybdate retention by soil and its uptake by sweet vernal.

Materials and Methods

The origin of the soil samples examined and the method of determination of oxalate-soluble molybdenum have been described previously (Grigg 1953, 1960).

The Distribution of Oxalate-soluble Molybdenum in Zonal Soils

When molybdenum levels for South Island soils are considered alongside those for North Island soils, where many are of volcanic origin, the overall picture for South Island soils is a more uniform, lower level of oxalate-soluble molybdenum. The results for zonal and associated skeletal soils, arranged in the same manner as in Part 1 of this series but with class intervals of 0.05 p.p.m., are given in Table 1 for the sub-divisions brown-grey earths, yellow-grey earths, yellow-brown earths, and podzolized yellow-brown earths and podzols.

(a) *Brown-grey Earths*

These semi-arid soils (13–20 in. rainfall), where chemical weathering is less important than physical weathering, are moderately well supplied with molybdenum, the overall mean for 49 samples being 0.13 p.p.m. The soil reaction is generally in the range pH 6–7, and field responses to molybdate top-dressing have not been obtained.

(b) *Yellow-grey Earths*

A generally apparent feature of the various sub-divisions of this soil group is that soils formed from sedimentary rocks of

Text continued on page 304

TABLE 1. ACID OXALATE-SOLUBLE MOLYBDENUM LEVELS OF ZONAL SOILS

Soil Group and Parent Materials † (Key in footnote)	Topography * (Key in footnote)	Soil Sets	Mean	S.D.	No. of Samples with Mo Level P.P.M. Range					Response of Pasture to Molybdate Top-dressing
					0-0.05	0.06-0.10	0.11-0.15	0.16-0.20	> 0.20	
B.G.E.										
Greywacke	M	Omarama	0.13	0.03	–	1	5	1	–	No responses
Greywacke alluvium	T	Acheron, Ranfurly	0.13	0.06	1	5	7	5	1	No responses
Schist	M	Arrow	0.13	0.13		–	3		-	No responses
Schist loess and alluvium	H	Blackstone, Clyde, Becks	0.15	0.03		3	4	7	1	No responses
Schist loess and gravels	T	Drybread	0.12	0.06		2	1	2		No responses
Y.G.E. with dry climate										
Greywacke loess	H	Timaru, Ngapara, Waipara, Ashley	0.11	0.05	1	20	10	4	1	Lucerne responses on Timaru
Greywacke loess and alluvium	TF	Lismore, Chertsey, Eyre, Dashwood, Glasnevin, Renwick	0.09	0.04	8	20	12	4		Good responses
Greywacke loess and alluvium	TI	Mairaki, Pukeuri	0.10	0.03		6	4	-		Lucerne responses on Mairaki
Greywacke and tertiary rocks	H	Seddon, Wither, Flaxbourne, Sedgemere	0.19	0.08	–	1	3	2	5	Wither responds
Mudstone	H	Ward	0.16	0.01	–	–	2	1	–	Slight responses
Tertiary sandstone	H	Kauru	0.11	0.04	1	6	7	2	1	Responses
Calcareous sandstone	H	Cheviot, Glendhu	0.13	0.07	–	1	3	1	–	No information
Y.G.E. with damp climate										
Greywacke	M	Weld	0.11	—		–	1	–	–	No information
Greywacke loess	H	Claremont, Opuha, Glenroy	0.09	0.06	2	15	6	–	–	Excellent responses
Greywacke loess alluvium	TF	Lyndhurst, Hororata	0.10	0.01	–	2	1		–	Slight responses
Greywacke loess and alluvium	TI	Dipton	0.16	0.09	1	1	–	1	1	No information
Schist loess	H	Clydevale, Te Houka	0.09	0.04	3	5	2	1	-	Excellent responses
Schist loess	TI	Wharetoa	0.07	0.03	2	2	1	-	–	No information
Greywacke and sandstone	M	Waihopai	0.19	—	–	–	–	2	–	No information
Alluvium from calcareous sandstone	TI	Motunau	0.08	0.01		2	–		–	Slight responses
Soils intermediate between Y.G.E. and Y.B.E.										
Greywacke	M	Hurunui	0.11	0.04	2	1	4	1	–	Some responses
Greywacke loess, alluvium, and colluvium	H	Kakahu, Charwell, Oxford, Okuku, Mossburn, Ohai, Tuamarina, Pawson	0.08	0.03	5	15	8	1	–	Most of these respond
Greywacke loess	T	Gorge, Mt. Somers, Ruapuna	0.07	0.04	2	7	1	-	–	All respond
Schist loess	H	Crookston, Waitahuna, Warepa, Waikokoi, Raurekau, Karitane	0.09	0.04	6	19	6	2	–	Excellent responses

Greywacke and schist loess	T	Waimumu	0.07	0.02	1	5	–	–	–	Responds
Basic greywacke loess	T	Pukemutu, Aparima	0.10	0.06	2	3	1	2	–	Pukemutu responds
Mudstone and sandstone	H	Kekerengu, Medway, Kahutara, Onepunga	0.15	0.06	–	3	5	3	5	Kekerengu responds
Sediments from greywack and tertiary rocks	T	Medina, Lottery, Jordan	0.13	0.05	1	5	6	1	1	Jordan responds
Y.B.E. moderately leached										
Greywacke alluvium and loess	T	Staveley	0.10	—	–	1	–	–	–	No information
Schist and schist loess	U	Wehenga	0.10	0.05	–	8	3	1	–	Responds
Schist and schist loess	H	Tuapeka	0.05	—	1	–	–	–	–	Responds
Basic greywacke loess	H	Kaihiku, Owaka	0.21	0.06			1	1	2	Responds
Tertiary silt and mudstones	H	Orawia	0.20	0.02	..		–	1	1	No information
Maitai shales	M	Whangamoa, Opouri	0.07	0.04	1	2				Responds
Moutere gravels	H	Stanley	0.04	—	3	–	–	–	–	No information
Alluvium from Moutere gravels	T	Motupiko	0.03	0.02	2	1	–	–		No information
Y.B.E. strongly leached										
Greywacke	M	Kaikoura	0.14	0.06	–	3	3	4	1	Generally responds
Greywacke (+ loess)	H	Cass, Tekoa, Chaslands	0.12	0.04		–	2	2	–	No information
Greywacke and granite alluvium	T	Ahaura	0.07	0.03	2	1	1	–		No information
Schist	M	Dunstan	0.16	0.04	–		2	2		No information
Basic greywacke and loess	H	Kaiwera	0.21	0.06	–	1	1	3	5	No information
Basic greywacke, loess, and alluvium	T	Waikiwi	0.14	0.03			1	1	–	No information
Calcareous mudstone	H	Tadmor, Runanga	0.18	0.02			–	1	1	No information
Tertiary mud and sandstones	H	Mangapiri, Lillburn	0.18	0.02		-		2	–	No information
Alluvium from Maitai shales	T	Rai	0.16	—		–	–	1	-	No information
Moutere gravels	H	Rosedale, Korere	0.03	0.01	4				–	Good responses
Y.B.E. strongly leached with patches of podzols										
Basic greywacke and loess	H	Waimahaka	0.17	0.07	–	1	1		2	No information
Basic greywacke and loess	T	Mokotua	0.17	0.06	–	–	2	1	2	No information
Loess over tertiary sediments	H	Orepuke	0.23	0.09	–	–	1	–	2	No information
Podzolized Y.B.E. and Podzols										
Greywacke and granite alluvium	H	Waiuta	0.04	–	1	–	–	–	–	No information
Greywacke and granite alluvium	T	Sergeant, Kumara, Okarito	0.03	0.02	3	-			–	No information
Basic greywacke	H	Tautuku	0.21	0.01	–	–	–	1	1	No information
Basic greywacke silts	T	Tisbury	0.15	0.08		2			1	No information
Alluvium from granite and mudstone	T	Kongahu	0.10	..		1				No information

† B.G.E.—Brown-grey Earth; Y.G.E.—Yellow grey Earth; Y.B.E.—Yellow-brown Earth.

* M—Mountains; U—Uplands; H—Hills and Downs; T—Terraces and Fans; F—Free drainage; I- Impeded drainage.

tertiary age (or derived materials) in Marlborough have higher mean values than Canterbury, Otago, and Southland soils. It is quite likely that this difference reflects a higher molybdenum content of the parent material of the Marlborough soils, but no total molybdenum figures are available to check this point.

This soil group can be considered in two divisions:—

(i) *Dry Climate.* The soils formed under a *dry climate* comprise a sub-group which, while frequently very low in oxalate-soluble molybdenum, is only moderately responsive to molybdate fertilisers. Responses have sometimes been greater on lucerne than on clover pasture. Lynch (1954) suggested that weakly weathered, weakly leached soils rarely respond to molybdenum; a number of responses have since been obtained, however.

(ii) *Damp Climate/Y.G.E.–Y.B.E. Intermediates.* The yellow-grey earths formed under a *damp climate* and the *soils intermediate between yellow-grey and yellow-brown earths* form the second sub-group. These soils have lower oxalate-soluble molybdenum levels than the brown-grey, dry yellow-grey, or yellow-brown earths. Covering large areas of downlands in Canterbury and Otago, they constitute the main region of molybdenum-responsive soils in the Dominion.

(c) *Yellow-brown Earths*

This group has been separated into four stages of leaching but presents a rather confused picture.

Levels in the *moderately* and *strongly-leached* groups are generally higher than in the yellow-grey earths. These higher levels may be related to the higher molybdate-retention power observed in these groups by Wells (1956). In the *podzolized yellow-brown earths* and *podzols* extremely low levels are encountered. There are some differences due to parent material, especially the occurrence of higher molybdenum levels on soils formed from basic greywackes in Southland and South Otago, and exceedingly low levels found in soils in the Nelson district derived from Maitai shales and Moutere gravels. Kidson (1954) pointed out that the latter soils have total molybdenum levels of less than 0.3 p.p.m. Oxalate-soluble molybdenum levels are all less than 0.05 p.p.m. Field reponses occur on these soils.

In Fig. 1 mean molybdenum levels for various parent materials have been plotted against soil group. Although the differences in molybdenum levels may not be statistically different, the graph does show that the soils on tertiary sediments and basic greywackes tend to have higher oxalate—soluble molybdenum levels than the other two soil groups. The increased molybdenum level found in moderately and strongly leached yellow-brown earths is also apparent. Wells found a similar trend for greywacke soils in his studies of molybdate retention.

Molybdenum Distribution in Azonal and Intra-zonal Soils

The results for these groups are listed in Table 2. The *recent soils from alluvium* are on the whole similar in soluble molybdenum

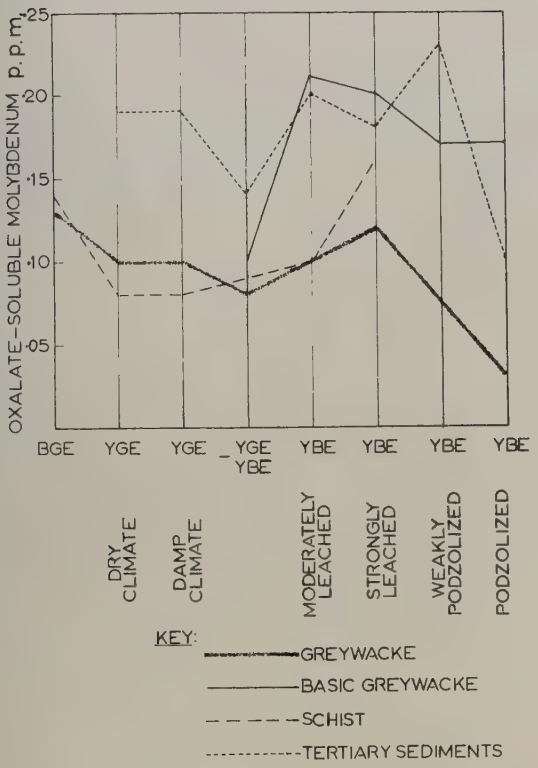

Fig. 1.—Distribution of molybdenum in zonal soil groups according to parent material.

content to the less developed zonal soils. An exception is the Wingatui soil of the Taieri plain, which is very high in molybdenum, in contrast with the Warepa soil of the surrounding hills, which is one of the most responsive soils known.

Gley soils contain relatively higher amounts of molybdenum than nearby alluvial or zonal soils. For example, Richmond gley soils average 0.21 p.p.m. compared with Ronga recent soil (0.10 p.p.m.) or Rai yellow-brown earth (0.16 p.p.m.).

The few examples of *soils of the coastal sand dunes are low,* reflecting the absence of clay minerals which would retain molybdate by adsorption.

There is a wide variation in the *rendzina and pedocal group of soils* formed on lime-rich rocks. Many more samples would be required to determine the reason for this variation.

Only a few *organic soils* have been analysed. Here, as in the North Island, molybdenum levels of acid peats such as the Invercargill

Text continued on page 308

TABLE 2. ACID OXALATE-SOLUBLE MOLYBDENUM LEVELS OF AZONAL AND INTRA-ZONAL SOILS

Soil Group and Parent Materials	Soil Sets	Mean	S.D.	No. of Samples with Mo Level P.P.M. Range					Response of Pasture to Molybdate Top-dressing
				0–0.05	0.06–0.10	0.11–0.15	0.16–0.20	> 0.20	
Recent to young soils									
Alluvium from greywacke	Waimakariri, Wairau, Omaka, Paparua, Templeton, Fairhall, Kaiapoi, Opawa, Mayfield, Tasman, Mataura	0.14	0.07	–	16	21	12	12	A few slight responses
Loess from greywacke	Barrhill, Kowai	0.12	0.03	–	2	2	–	–	No information
Alluvium from schist	Idaburn, Clutha, Koromiko	0.14	0.06	–	6	9	5	3	No information
Alluvium from greywacke, granite and schist	Hokitika, Ikamatua, Karamea	0.14	0.04	–	10	9	11	1	No information
Alluvium from greywacke and greensand	Willowbridge	0.13	—	·	·	1	·	·	No information
Alluvium from schist, basic volcanic rocks, and sandstones	Wingatui	0.45	0.15	–	–	–	–	8	No responses
Alluvium from basic greywacke, diorite, and granite	Tuatapere	0.21	0.07	–	–	1	1	1	No information
Alluvium from Maitai shales	Ronga	0.10	0.06	2	2	1	1	–	No information
Alluvium from Maitai shales, Moutere gravels, and basic greywackes	Waimea	0.10	0.04	2	1	7	–	–	No information
Gley soils									
Alluvium from greywacke	Taitapu, Temuka, Waterton, Spring Creek, Paynter	0.20	0.16	–	4	4	4	6	Lucerne responses on Temuka
Alluvium from basic greywacke	Makarewa	0.17	0.03	–	–	1	3	–	No information
Alluvium from schist, greywacke and granite	Harihari, Karangarua	0.22	0.12		2	1	-	1	No information
Alluvium from Maitai shales and Moutere gravels	Richmond	0.21	—	–	–	–	–	1	No information
Saline Gley soils									
Alluvium from greywacke	Motukarara	0.13	0.05	1	1	3	3	–	No information

Soils of coastal sand dunes									
Sands from greywacke	Taumutu, Kairaki, Utopia	0.07	0.04	3	2	1	–	–	No information
Sands from greywacke, granite, and schist	Otatara, Mahinapua	0.09	0.02		3	1	–	–	No information
Soils formed on lime-rich rocks									
Limestone, Marble	Amuri, Pikikuranga	0.14	0.09	–	4	1	1	2	No information
Limestone and loess	Oamaru	0.25	0.09	–	–	1	–	4	No response
Sandy limestones and marls	Hui Hui	0.06	0.01	1	1	–	–	–	No information
Calcareous sandstones and mudstones	Heslington, Kauana, Te Mara	0.14	0.06	–	2	5	–	2	No information
Soils formed on basic rocks (igneous and sedimentary)									
Diorite	Kawatiri	0.05	—	1	–	–	–	–	No information
Basalt ash, limestone, and loess	Waiareka	0.14	0.02	–	–	1	1	–	No information
Basic greywackes and basalt tuff	Malakoff	0.29	0.05	–	–	–	–	2	No information
Alluvium from basic greywackes	Drummond, Wairaki	0.16	0.06	–	1	2	2	1	No information
Organic soils									
Organic matter from rushes and moss	Otanomomo	0.07	0.03	1	1	–	–	–	No information
Organic matter from sedges, rushes, flax, and silt and clay	Waimairi, Waitapeka, Windermere	0.32	0.14	–	–	–	1	4	No information
Acid peat	Invercargill	0.09	0.04	–	4	1	–	–	No information

soil are much lower than those of the mellow peaty loams such as the Waimairi soils.

The soils catalogued in Tables 1 and 2 include only samples from specific named soil types. Many other analyses have been carried out on samples of soils not yet classified. Where these samples can be associated with a soil group, they generally fall within the range found for that group.

The situation of small differences between groups in the South Island contrasts strongly with that of larger differences found in the North Island, and points to the dominant role of parent material in determining the molybdenum status of South Island soils.

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THE MINERAL REQUIREMENTS OF RADIATA PINE SEEDLINGS

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Summary

Radiata pine seedlings were grown in water cultures and perlite cultures in which N, P, K, and Mg were supplied at several rates. It was found that deficient or excess supplies of most nutrients cause chlorosis. The blue-green foliage on P-deficient seedlings is an exception. The patterns of chlorosis produced by N, K, and Mg deficiencies are distinct and characteristic.

Quantities of 100 p.p.m. of N, 1 p.p.m. of P, 10 p.p.m. of K, and 10 p.p.m. of Mg in the nutrient solution are sufficient to maintain good growth in seedlings. These levels are low when compared with those obtained in some other studies, but they are of the same order as the concentrations found in the soil solutions of two New Zealand soils known to support vigorous plantations of radiata pine. In an experiment of this nature it is important to have the basic nutrient concentrations similar to levels found in soil solutions, otherwise optimum levels of supply and critical foliage levels may not apply to plants grown under field conditions.

Foliage contents of 1.6% of N, 0.10% of P, 1.1% of K, and 0.11% of Mg indicate adequate supplies of each nutrient.

The excess or deficient supply of one nutrient causes characteristic changes in the uptake of other nutrients.

Introduction

Although radiata pine (*Pinus radiata* D. Don) has only a very restricted natural range along the California coastline, it is proving to be an adaptable and very productive species in many countries, especially in the temperate zone of the southern hemisphere. Planted on widely differing sites, it has been found in some cases to suffer from mineral deficiencies. In Australia applications of phosphorus and zinc to the soil have corrected deficiencies of these elements (Kessell and Stoate 1938; Ludbrook 1939; Stoate 1950; Young 1940), and some work has been carried out on the trace-element requirements of seedlings (Ludbrook 1942; Smith 1943; Smith and Bayliss 1942). In New Zealand, too, the growth of trees in some forests is seriously affected by phosphate deficiency (Weston 1956, 1958).

Apart from some recent work by Purnell (1958), the effects of major element deficiencies on the growth of radiata pine have not been

studied under controlled conditions. Morrison (1957) reported the influence of mycorrhizae on the uptake of phosphorus, and other analyses on seedlings have been carried out by Askew (1937), Voigt (1955), and Mader (1959). Some variations in the mineral composition of needles of adult trees have been considered by Will (1957). However, only in a study by Travers (1959) have the vigour and growth of seedlings been correlated with nutrient supply. Studies designed to correlate growth with nutrient supply and foliage composition have been carried out on many other forest tree species, e.g., *Pinus strobus* (Mitchell 1939), *Thuja plicata* (Walker *et al.* 1955), *P. taeda* and *P. virginiana* (Fowells and Krauss 1959), *P. sylvestris* (Leyton and Armson 1955; Tamm 1956; Boszormenyi 1958), *Larix leptolepis* (Leyton 1956, 1957; van Goor 1953), *Pseudotsuga taxifolia* (Gessel *et al.* 1951), *Picea abies* (Ingestad 1959), and *Betula verrucosa* (Ingestad 1957).

As radiata pine will be relied on for the foreseeable future for the major part of New Zealand's timber production, a knowledge of its nutrient requirements is of some importance. A series of investigations is being carried out, and this paper reports the results obtained by varying the supplies to seedlings of nitrogen, phosphorus, potassium, and magnesium.

Experimental Procedure

Nitrogen was supplied to seedlings at different rates while keeping the supply of other nutrients constant. In this way the effects of deficiency and excess levels of supply on the growth rate, appearance, and chemical composition of seedlings were followed. Similarly the supplies of phosphorus, potassium, and magnesium were varied one at a time. In addition, seedlings were grown in solutions containing high and low concentrations of all the major elements in the complete nutrient solution (i.e., N, P, K, Mg, Ca, and sulphur). The supply of trace elements was constant for all treatments.

After preliminary trials two series of experiments were carried out. One was in water culture, so that root development could be studied; these seedlings were grown for 4½ months (August–January), and reached heights of up to 7 in. The other series was in perlite, a suitable nutrient-free rooting medium (Morrison *et al.* 1960). These "sand" cultures were grown for a full season (eight months, October–May).

All seedlings were grown in the open. Clear plastic covers were used, to provide protection against the rain yet to keep temperature and humidity as close as possible to field conditions and so obtain a similar rate of growth. It has been found that even in an unheated glass-house radiata pine seedlings produced soft, lank growth.

The seed used was lot number FRI/58/967, seed collected from selected trees in Kaingaroa Forest. The complete nutrient solution used was made up with reagent-grade salts and de-ionised water to

the formula of Walker *et al.* (1955). Where lower levels of a cation were used, sodium salts were added to keep the molarity constant, and when an anion was lowered chloride was used as a replacement. Similarly, higher levels were added as chlorides or sodium salts. Iron in the form of $FeCl_3$ was added to the water-culture experiments and initially to the perlite cultures. Later the perlite cultures received iron as Fe—EDTA.

Water-culture Series

Each treatment consisted of seven seedlings grown in a 2 litre glass beaker of nutrient solution. The beakers had been painted black on the outside and then silver. Seedlings were supported, by wads of cotton wool, in holes in a covering piece of wax-impregnated hardboard. After germination on damp filter paper seedlings were "planted out," and for the first week solutions were kept at half strength. Thereafter full-strength solutions were used, and were changed completely each week.

Because of practical difficulties, solutions were not aerated, but a side experiment showed that no beneficial effects followed aeration. Walker *et al.* (1955) obtained a similar result.

Height measurements and descriptions of both roots and shoots were made at fortnightly intervals. At the age of 4½ months seedlings from each beaker were harvested, divided into roots and shoots, dried, weighed, and analysed.

Perlite-culture Series

Each treatment consisted of two plastic pots (capacity approx. 1½ litre) filled with acid-washed perlite and planted with five newly germinated seedlings. After a few weeks numbers were reduced to three evenly-sized seedlings per pot. Pots were watered three times a week with excess nutrient solution to flush the whole rooting medium each time.

Height measurements and descriptions were made at fortnightly intervals once growth differences became apparent. At the age of eight months the seedlings from each treatment were harvested and the roots washed free of perlite. After dividing into roots and shoots, seedlings were dried, weighed, and analysed.

Methods of Analysis

Major elements were determined by the methods of Metson (1956). Calcium and magnesium were estimated using versenate titrations; potassium and sodium using a flame photometer; nitrogen by a micro-Kjeldahl technique; and phosphorus colorimetrically by the method of Hanson (1950). Iron and manganese were estimated in the acid ash extract, using thioglycollic acid and leucomalachite green respectively.

Results and Discussion

The effects of variations in the supply of individual nutrients are discussed first; the overall implications of the results are considered later in the paper.

NITROGEN

Deficiency Symptoms

Shoots. Foliage is a uniform yellowish green, becoming pale yellow under severe conditions (see Purnell 1958).

Roots. (In water culture). Longer and greater development than normal; short side roots very short; all roots thinner and browner than normal.

Excess Symptoms

Chlorosis of needle tips followed by necrosis.

Fig. 1.—Representative seedlings from the series of water cultures containing different concentrations of nitrogen. From left to right: 595 p.p.m., 105 p.p.m., 1.4 p.p.m., 1.0 p.p.m., and 0.5 p.p.m.

Growth Rates and Mineral Composition

The results from the growth of seedlings in water culture (see Fig. 1) are given in Table 1; results from growth in perlite appear in Table 2. Fowells and Krauss (1959) concluded that loblolly and Virginia pines grow best when N is supplied at 25–100 p.p.m. From these water and perlite culture results it seems that radiata pine, too, requires an N supply at about 100 p.p.m. for good growth; below this level shoot growth fell off gradually but the weight of roots remained fairly constant.

TABLE 1. DIFFERENCES IN HEIGHT, WEIGHT, AND COMPOSITION OF RADIATA PINE SEEDLINGS WITH VARIATION IN THE SUPPLY OF NITROGEN. GROWN IN WATER CULTURE FOR 4½ MONTHS.

P.p.m. of N in Soln.	Height (in.)	Wt (g)	N %	P %	K %	Ca %	Mg %	Na %	Fe %	Mn (p.p.m.)	Wt of N (mg)
					SHOOTS						
0.0	1.1 SV	ND	1.1	0.59	1.8	0.00	0.11	0.18	ND	ND	ND
0.5	3.2 SV	0.37	1.5	0.63	1.9	0.11	0.24	0.02	0.008	420	5.6
1.4	4.2 V	0.49	1.2	0.53	1.6	0.13	0.20	0.03	0.009	420	5.6
105.0	**5.8**	**1.13**	**1.6**	**0.24**	**1.2**	**0.13**	**0.17**	**0.03**	**0.004**	**280**	**18.0**
595.0	6.0 V	0.81	2.5	0.32	1.3	0.06	0.11	2.63	0.006	300	20.4
					ROOTS						
0.0		ND	1.0	0.77	1.4	0.00	0.10	0.19	0.6	ND	ND
0.5		0.21	0.9	1.09	2.6	0.20	0.13	0.14	0.6	260	1.8
1.4		0.24	1.1	1.13	3.1	0.16	0.13	0.14	0.7	340	2.5
105.0		**0.19**	**2.4**	**0.92**	**0.9**	**0.20**	**0.38**	**0.02**	**1.4**	140	**4.6**
595.0		0.16	1.1	0.95	2.2	0.13	0.20	2.70	1.2	1050	1.8

V—Visual symptoms of nutrient imbalance present other than decreased growth.
SV—Severe visual symptoms present. ND—Not determined.
Results in bold type from complete nutrient solution.

TABLE 2. DIFFERENCES IN HEIGHT, WEIGHT, AND COMPOSITION OF RADIATA PINE SEEDLINGS WITH VARIATION IN THE SUPPLY OF NITROGEN. GROWN IN PERLITE CULTURE FOR 8 MONTHS.

P.p.m. of N in Soln.	Height (in.)	Wt (g)	N %	P %	K %	Ca %	Mg %	Na %	Fe %	Mn (p.p.m.)	Wt of N (mg)
					SHOOTS						
9.7	5.6 SV	1.1	1.3	0.54	2.2	0.06	0.02	0.01	0.007	265	1.4
19.3	7.5 V	1.8	1.2	0.34	1.7	0.16	0.18	0.16	0.009	120	2.1
105.0	**15.0**	**8.5**	**1.8**	**0.24**	**2.3**	**0.39**	**0.17**	**0.08**	**0.005**	**210**	**15.1**
					ROOTS						
9.7		0.85	0.8	0.43	1.5	0.17	0.18	0.54	0.05	240	0.7
19.3		0.97	0.9	0.38	1.8	0.87	0.34	0.47	0.07	170	0.9
105.0		**2.60**	**1.7**	**0.24**	**1.5**	**0.23**	**0.19**	**0.60**	0.09	**360**	**4.5**

With decreasing N supply the percentage of N in the roots decreased, but in the shoots an initial decrease was followed by an increase. Even in seedlings grown in a nutrient solution containing no added N the percentage of N in the foliage was more than 2/3 that in seedlings supplied with adequate N. The total N in the shoots, was, of course, much smaller because of a very low oven-dry weight. However, this relatively high percentage of N in very deficient seedling shoots makes it difficult to define a percentage of N in the shoots below which growth is restricted. It would seem that about 1.6% of N is sufficient provided that no obvious chlorosis is present.

Much lower percentages of N have been found in foliage samples from the field. Reduced percentages probably result from fluctuating N supplies; if deficient conditions follow an adequate supply, the plant will attempt to maintain its growth rate at the expense of the N already held in the foliage. Some N may even be used in increased root growth when a deficiency develops. The following figures illustrate the low level that can be found in the foliage of seedlings in the field:

	N %	P %	K %
Regeneration seedlings growing in normal soil	2.5	0.11	1.13
Similar-aged chlorotic seedlings growing on old loading bay	0.9	0.36	0.78

The percentage of N in the foliage of seedlings growing on the old loading bay was very low, owing to the lower N supply caused by: (1) the removal of the topsoil; and later by (2) removal of available N by decomposing bark and wood. The figures just quoted also confirm those in Tables 1 and 2 where, when N was deficient, the percentage of P in the shoots was greater than normal.

An excess N supply did not decrease height growth, but weights of shoots and roots did decrease. The percentage of N in the shoots increased, but there was no similar rise in the roots. The percentage of Na in both shoots and roots increased greatly, but this may have been due to an increased Na supply, part of the excess N being supplied as $NaNO_3$. The high Mn concentration in the roots cannot be accounted for, but it should be noted that an even higher value (1,800 p.p.m.) was found for the roots of seedlings grown in the complete nutrient solution at five times normal strength. (This is discussed later.)

PHOSPHORUS

Deficiency Symptoms

Shoots. Foliage darker green than normal, tending to a blue-green (see Purnell 1958). As height growth decreases in young seedlings primary leaves are shorter and set closer together, giving a tuft of leaves at the top of the stem—*cf.* symptoms in adult trees (Weston 1956). It should be noted that P deficiency in adult trees in the field results in lighter coloured foliage with chlorotic needle tips in many cases.

Roots. Similar to N deficiency, i.e., longer, thinner, and browner than normal, with short side roots.

Excess Symptoms

Needles on tip of stem and tips of side branches a yellowish green.

Growth Rates and Mineral Composition

Table 3 gives the results from the series of water cultures in which the level of P was varied. No figures are reported for perlite cultures, as difficulty was experienced in getting a range of growth

TABLE 3. Differences in Height, Weight, and Composition of Radiata Pine Seedlings with Variation in the Supply of Phosphorus. Grown in Water Culture for 4½ Months.

P.p.m. of P in Soln.	Height (in.)		Wt (g)	N %	P %	K %	Ca %	Mg %	Na %	Fe %	Mn (p.p.m.)	Wt of P (mg)
						SHOOTS						
0.0	1.5	SV	ND	2.9	0.06	1.3	0.12	0.13	0.09	ND	ND	ND
0.23	2.5	SV	0.4	2.5	0.06	1.2	0.28	0.17	0.02	0.005	450	0.3
0.62	5.6	V	0.9	1.9	0.09	1.4	0.16	0.13	0.01	0.004	240	0.8
0.93	7.0		1.3	1.7	0.14	1.4	0.17	0.15	0.03	0.004	290	1.8
15.5	**5.8**		**1.1**	**1.6**	**0.24**	**1.2**	**0.13**	**0.17**	**0.03**	**0.004**	**280**	**2.7**
170.0	5.4	V	1.0	1.7	0.51	1.2	0.03	0.13	0.77	0.001	300	4.9
						ROOTS						
0.0			ND	2.0	0.09	1.3	0.04	0.10	0.09	1.8	ND	ND
0.23			0.21	2.1	0.20	2.3	0.28	0.17	0.07	1.3	15	0.4
0.62			0.24	2.4	0.45	2.0	0.24	0.32	0.07	0.8	80	1.1
0.93			0.26	2.4	0.28	2.2	0.22	0.31	0.09	0.7	80	0.7
15.5			**0.19**	**2.4**	**0.92**	**0.9**	**0.20**	**0.38**	**0.02**	**1.4**	**140**	**1.8**
170.0			0.23	2.7	0.90	1.2	0.22	0.17	0.75	1.1	280	2.1

rates. It does seem that generally higher rates of P may be needed in perlite cultures.

With a decreasing supply of P, the percentage of P in the shoots decreased more rapidly than that in the roots, and it seems that while 0.14% of P in the shoots indicates an adequate supply, 0.09% of P indicates deficiency. These figures are very similar to those found for loblolly and Virginia pines by Fowells and Krauss (1959), who also reported that a constant supply of 1 p.p.m. of P in the solution was sufficient to maintain normal growth. Radiata pine again reacted similarly. (See Fig. 2.) Woodwell (1958) reported values of 40–600 p.p.m. for the best growth of loblolly and pond pines, but the data presented do allow for a lower concentration being sufficient for optimum growth.

These figures for the P content of seedling foliage also show the same ranges as those for the foliage of adult trees. In connection with superphosphate trials in Riverhead Forest (see Weston 1956, 1958) foliage analyses have been carried out for several years. Full results will be published later, but indications are that 0.10% of P in the current year's foliage is on the borderline between a sufficient and a deficient P supply.

It has been seen that a decreasing N supply was accompanied by a higher percentage of P in the shoots than normal; similarly a decreasing P supply was accompanied by an increasing percentage of N in the shoots.

Mention has been made of the high Mn values for roots when the N supply was in excess. When the P supply was deficient the level of Mn in the roots fell but, as in the case of excess N supply, the Mn concentration in the shoots remained normal.

Fig. 2.—Representative seedlings from the series of water cultures containing different concentrations of phosphorus. From left to right: 170 p.p.m., 15.5 p.p.m., 0.9 p.p.m., 0.6 p.p.m. and 0.23 p.p.m.

Excess P supply increased the percentage of P in the shoots but not in the roots; it should be noted, however, that at all levels of supply the concentration of P was greater in the roots than in the shoots. Excess P supply resulted in high Na figures but, as in the case of excess N, the Na supply was high.

POTASSIUM

Deficiency Symptoms

Shoots. In young seedlings severe deficiency produces red patches on primary leaves, especially near the tips. In older seedlings needles become chlorotic. The chlorosis is more pronounced on the tips of needles (in contrast with N deficiency) but is not entirely absent from the remaining length of needles; (this is in contrast with Mg deficiency; see later, and Purnell 1958). Chlorotic symptoms are intensified if Fe is in short supply. The supply of Fe-EDTA greatly reduced chlorosis in perlite-culture K-deficient seedlings.

Roots. There is no elongation of roots as in N and P deficiency. Side roots are thicker than normal.

Excess Symptoms

Needle-tip chlorosis and burning, especially near the base of stem.

TABLE 4. Differences in Height, Weight, and Composition of Radiata Pine Seedlings with Variation in the Supply of Potassium. Grown in Water Culture for 4½ Months.

P.p.m. of K in Soln.	Height (in.)	Wt (g)	N %	P %	K %	Ca %	Mg %	Na %	Fe %	Mn (p.p.m.)	Wt of K (mg)
					SHOOTS						
0.0	1.7 SV	ND	4.0	0.45	0.2	0.05	0.09	0.67	ND	ND	ND
1.1	5.0 V	0.8	2.3	0.28	0.5	0.04	0.19	0.40	0.002	290	3.7
3.5	5.7 V	1.4	1.7	0.16	0.7	0.15	0.17	0.72	0.003	140	9.4
8.2	6.3	0.9	1.8	0.28	1.1	0.15	0.14	0.34	0.002	360	10.4
117.0	**5.8**	**1.1**	**1.6**	**0.24**	**1.2**	**0.13**	**0.17**	**0.03**	**0.004**	**280**	**13.0**
1,287.0	4.4* V	0.8	2.0	0.26	6.2	0.09	0.19	0.90	0.004	210	48.6
					ROOTS						
0.0		ND	3.7	0.87	0.2	0.00	0.10	0.61	0.5	ND	ND
1.1		0.19	3.2	0.86	0.8	0.25	0.27	0.99	1.2	360	1.4
3.5		0.21	2.4	0.66	0.4	0.24	0.29	0.43	1.0	260	0.9
8.2		0.27	2.2	0.99	1.8	0.20	0.20	1.21	1.1	350	4.4
117.0		**0.19**	**2.4**	**0.92**	**0.9**	**0.20**	**0.38**	**0.02**	**1.4**	**140**	**1.6**
1,287.0		0.14	3.0	0.38	2.6	0.36	0.12	0.14	0.9	100	3.6

* Seedlings harvested 2 weeks early owing to poor condition: 'complete' seedlings 5.3 in. high.

TABLE 5. Differences in Height, Weight, and Composition of Radiata Pine Seedlings with Variation in the Supply of Potassium. Grown in Perlite Culture for 8 Months.

P.p.m. of K in Soln.	Height (in.)	Wt (g)	N %	P %	K %	Ca %	Mg %	Na %	Fe %	Mn (p.p.m.)	Wt of K (mg)
					SHOOTS						
2.3	9.0 V	3.1	1.6	0.30	0.4	0.53	0.22	0.26	0.006	250	33.8
9.2	9.3 V	2.7	1.8	0.32	0.9	0.36	0.23	0.27	0.006	360	24.9
117.0	**15.0**	**8.5**	**1.8**	**0.24**	**2.3**	**0.39**	**0.17**	**0.08**	**0.005**	**210**	**192.0**
					ROOTS						
2.3		1.4	1.4	0.33	0.4	0.33	0.32	0.76	0.04	190	4.9
9.2		1.0	1.2	0.34	0.2	0.43	0.32	0.73	0.04	225	1.9
117.0		**2.6**	**1.7**	**0.24**	**1.5**	**0.23**	**0.19**	**0.60**	**0.09**	**360**	**39.0**

Growth Rates and Mineral Composition

Variations in growth rates and chemical compositions resulting from different levels of K supply are given in Tables 4 and 5. The percentage of K in the roots of the water culture 'Complete' seedlings appears to be an erroneous result. Unfortunately, there was insufficient sample to do repeat analyses on this and many other of the root samples. From the water-culture results it seems that about 5–10 p.p.m. of K in the solution is adequate (see Fig. 3) and that the critical foliage level lies between 0.7% and 1.1% of K. However, the perlite-culture results suggest a need for a greater

Fig. 3.—Representative seedlings from the series of water cultures containing different concentrations of potassium. From left to right: 117 p.p.m., 8.2 p.p.m., 3.5 p.p.m., and 1.1 p.p.m.

supply of K. This could be due to a greater demand by older plants or it could be an effect from the rooting medium.

The critical foliage level of between 0.7% and 1.1% of K found in the water-culture series agrees with the few field observations that are available. In one forest chlorotic seedlings contained 0.5% of K, while nearby apparently healthy seedlings growing in a better soil contained 0.9% of K or more. These were seedlings from natural regeneration. In a nursery, small and chlorotic seedlings contained 0.3% of K and healthy seedlings in another part of the nursery 1.2% of K.

As the level of K in the solution supplied to the water and perlite cultures decreased, so did the percentage of K in the shoots and roots. The decrease in the foliage percentage of K was accompanied by an increase in the percentage of Na. This is but another example of Na at least partially replacing K when it is deficient. The excess supply of K resulted in a much higher percentage of K in both roots and shoots. As mentioned above, this luxury consumption was accompanied by yellowing and burning of the foliage. It seems that these symptoms were due to the excess K itself rather than to an induced deficiency of another nutrient. Why a high percentage of K should be associated with a high percentage of Na, when no Na was added to the nutrient solution, is not understood, although it is noticeable, from the results of this series of experiments, that many cases of nutrient imbalance (both deficiencies and excesses) resulted in high Na concentrations in the plant.

With K deficiency and lower levels of K in the shoots, levels of N and P rose. This was probably a similar case to higher K and P levels when N was deficient and a higher N level when P was deficient. These deficiencies, although retarding growth, did not impede the uptake of other nutrient elements, which therefore tended to accumulate.

MAGNESIUM

Deficiency Symptoms

Shoots. Needle-tip chlorosis followed by necrosis. Chlorosis more golden than with N or K deficiency (see Purnell 1958) and confined to needle tips. Chlorotic primary leaves at top of stem of young seedlings curl backwards soon after formation.

Roots. More extensive system than normal, otherwise similar.

TABLE 6. Differences in Height, Weight and Composition of Radiata Pine Seedlings with Variation in the Supply of Magnesium. Grown in Water Culture for 4½ Months.

P.p.m. of Mg in Soln.	Height (in.)	Wt (g)	N %	P %	K %	Ca %	Mg %	Na %	Fe %	Mn (p.p.m.)	Wt of Mg (mg)
					SHOOTS						
0.9	4.7 SV	0.8	2.3	0.36	3.4	0.07	0.12	0.20	0.003	350	0.9
1.8	5.7 V	1.0	1.8	0.28	1.5	0.10	0.09	0.25	0.002	300	0.9
2.8	6.2 V	1.2	1.9	0.25	1.4	0.09	0.08	0.28	0.003	240	1.0
23.0	**5.8**	**1.1**	**1.6**	**0.24**	**1.2**	**0.13**	**0.17**	**0.03**	**0.004**	**280**	**1.9**
253.0	7.2	1.1	1.6	0.26	1.4	0.13	0.61	0.32	0.005	190	6.5
					ROOTS						
0.9		0.20	1.7	2.1	2.6	0.08	0.07	0.67	1.1	800	0.1
1.8		0.22	2.7	1.1	1.8	0.10	0.20	0.50	1.0	215	0.4
2.8		0.24	2.9	2.1	2.2	0.26	0.07	0.60	1.1	240	0.2
23.0		**0.19**	**2.4**	**0.9**	**0.9**	**0.20**	**0.38**	**0.02**	**1.4**	**140**	**0.7**
253.0		0.19	2.2	2.2	2.7	0.04	0.75	0.09	0.9	190	1.4

TABLE 7. Differences in Height, Weight, and Composition of Radiata Pine Seedlings with Variation in the Supply of Magnesium. Grown in Perlite Culture for 8 Months.

P.p.m. of Mg in Soln.	Height (in.)	Wt (g)	N %	P %	K %	Ca %	Mg %	Na %	Fe %	Mn (p.p.m.)	Wt of Mg (mg)
					SHOOTS						
0.5	11.1 SV	5.6	1.7	0.27	2.7	0.61	0.06	0.06	0.004	145	3.4
1.9	12.0 V	6.6	1.6	0.27	2.5	0.45	0.09	0.26	0.002	140	5.9
24.0	**15.0**	**8.5**	**1.8**	**0.24**	**2.3**	**0.39**	**0.17**	**0.08**	**0.005**	**210**	**14.4**
					ROOTS						
0.5		1.9	1.7	0.18	1.0	0.66	0.08	0.62	0.04	90	1.5
1.9		2.1	1.5	0.22	1.4	0.72	0.11	0.86	0.04	80	23.1
24.0		**2.6**	**1.7**	**0.24**	**1.5**	0.23	**0.19**	**0.60**	**0.09**	**360**	**49.5**

Excess Symptoms

No pronounced differences from normal seedlings have been observed.

Growth Rates and Mineral Composition

Variations in the growth and mineral composition of seedlings resulting from different levels of Mg supply are given in Tables 6 and 7 (see also Fig. 4). The maximum growth rate in water culture occurred in the 2.8 p.p.m. Mg solution, but these seedlings showed some chlorosis; also their shoot level of 0.08% of Mg definitely represents deficiency conditions as found in nursery seedlings (Will 1961). From all available results it seems that a level of about 0.11% of Mg in foliage represents a sufficient supply, and this can probably be achieved with about 10 p.p.m. Mg in the nutrient solution.

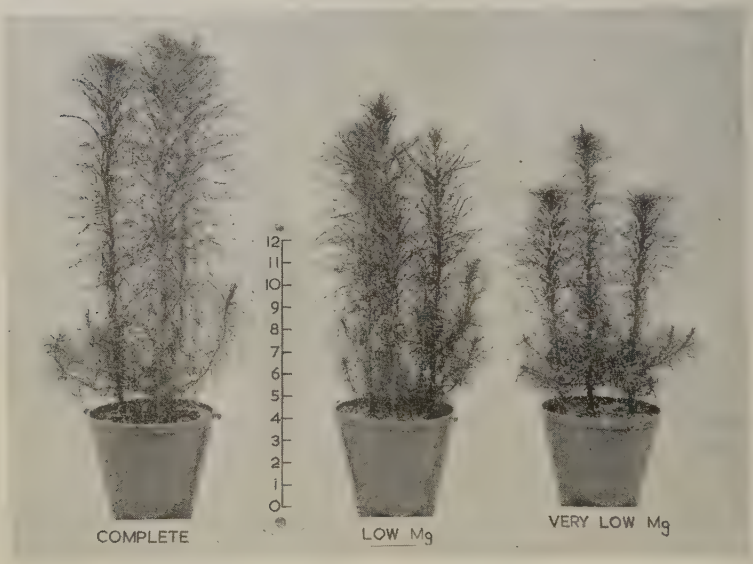

Fig. 4.—Representative pots from the series of perlite cultures receiving solutions with different concentrations of magnesium. From left to right: 24 p.p.m., 1.9 p.p.m., and 0.5 p.p.m. (Scale in inches.)

In the perlite culture the percentage of Ca in the seedlings increased as the percentage of Mg decreased. This rise in percentage of Ca did not occur in the water-culture seedlings, where levels of Ca were much lower. This difference in Ca levels indicates the effect of age on the Ca concentration in seedlings. It has already been shown that for radiata pine, as for other plants, the calcium contents of leaves, bark, and wood rise as the tissue ages (Will 1957; Orman and Will 1960).

With decreasing levels of Mg in the foliage there was an increase in the percentage of N, P, and K present, and this parallels previously mentioned examples of high uptake of some nutrients when another is deficient. The excess supply of Mg had little effect on the composition of the shoots except to raise the percentage of Mg present.

Variations in Strength of Complete Nutrient Solution

Seedlings were grown in solutions in which the concentrations of the major elements were 1/5 and 5 times the normal. The only visible effect on seedling growth was some chlorosis in the 5 solution; the terminal needles in particular were at times a very pale greenish yellow. As can be seen from Table 8, height growth was unaffected, but shoot dry weights were less in both the 1/5 and 5 solutions. The weight of roots was also lower in the 5 solution.

TABLE 8. Differences in Height, Weight, and Composition of Radiata Pine Seedlings with Variation in Concentration of Complete Nutrient Solution. Grown in Water Culture for 4½ Months.

Conc. of major elements in Soln.	Height (in.)		Wt (g)	N %	P %	K %	Ca %	Mg %	Na %	Fe %	Mn (p.p.m.)
						SHOOTS					
1/5 Normal	5.7		0.8	1.7	0.31	1.4	0.08	0.15	0.07	0.004	370
Normal	**5.8**		**1.1**	**1.6**	**0.24**	**1.2**	**0.13**	**0.17**	**0.03**	**0.004**	**280**
5 Normal	5.7	V	0.9	2.9	0.32	3.4	0.44	0.16	0.05	0.005	290
						ROOTS					
1/5 Normal			0.20	2.0	0.65	2.8	0.18	0.28	0.01	0.4	200
Normal			**0.19**	**2.4**	**0.92**	**0.9**	**0.20**	**0.38**	**0.02**	**1.4**	**140**
5 Normal			0.14	2.7	0.84	3.1	0.48	0.78	0.01	0.6	1800

Also in Table 8 are the figures for the mineral composition of shoots and roots. Ca levels were governed by the strength of the solution. Mg in the roots increased with increased supply but the level in the shoots was unaffected. N, P, and K percentages in the shoots were higher in both the 1/5 and 5 solutions. There were other variations in the composition of the shoots and roots but at this stage their significance is unknown. The only variation to be considered further is the high Mn level in the roots of the 5 seedlings. As mentioned earlier, a high figure also resulted from an excess supply of N. It should be mentioned too that the pale chlorosis observed in the 5 seedlings was very similar to the chlorosis that can be induced by an oversupply of Mn.

General Discussion

The levels of supply of N, P, K, and Mg which have been found to give good growth are of interest in themselves but are of limited practical value for several reasons. The determination of

TABLE 9. Comparison of the Composition of the "Complete" Nutrient Solution Used in Tree Nutritional Studies with the Optimum Concentrations Found for Individual Nutrients.

Tree Species		Composition of "Complete" Solution (p.p.m.)				Suggested Optimum rates of supply (p.p.m.)			
		N	P	K	Mg	N	P	K	Mg
Radiata pine	Present paper	105	15	117	23	100	1	10	10
Loblolly and Virginia pines	Fowells and Krauss	100	100	130	24	25-100	1	—	—
Birch	Ingestad	140	100	175	48	140	3-100	60	5
Spruce		50	10	50	15	50	10	50	15
White pine	Mitchell	300	235	319	176	300	350	150	—
Radiata pine	Travers	Based on Mitchell				230	300	100	—

concentrations of nutrients in the soil solution is difficult, and these concentrations change with varying moisture status and microbiological activity. Also, the uptake of some nutrients is more efficient under field conditions, owing to the presence of mycorrhizae. (Some dichotomously branched roots were present on seedlings in the water cultures but as they were without fungal mantles it can be said that nutrient absorption was direct from the medium in both series). Boszormenyi (1958) has shown that, for Scots pine, the optimum level of supply of any one nutrient varies greatly with the levels of supply of other nutrients. Further evidence of this can be seen in Table 9. Here the compositions of the nutrient solutions used in six studies are compared with the optimum rates found. Where the total concentration of all nutrients was relatively low, much lower optimum levels of supply were found. It seems, therefore, that ratios between the concentration of one nutrient and others are more important than absolute concentration in determining whether the supply to the plant is sufficient. Factorially designed experiments such as those carried out by Boszormenyi (1958) and Travers (1959) are the only kind that can give a full answer. However, the work involved in testing the effects of a wide range of concentrations of all nutrients is very considerable. From a practical point of view it does seem that experiments should cover the range of concentrations found in forest-soil solutions, but it appears that little information on this subject has been published.

Woodwell (1958) carried out experiments, with loblolly and pond pines, similar to those listed in Table 9, but this work has not been included as nutrient concentrations were not constant throughout the experiments. In addition, Woodwell reported soil analyses, and concluded that "the concentration of phosphate in the soil solution of even the best soils for pond pine in this study is probably seldom over a few

parts per million and usually less than one part per million". Stoate (1950) found that total soil P levels of 150 p.p.m. and 75 p.p.m. were sufficient for good growth of radiata pine and *Pinus pinaster* respectively. In Kaingaroa Forest, New Zealand, where the rate of growth of radiata pine is high, the soil contains about 200 p.p.m. total P. Of this total only a small amount is in the soil solution. T. W. Collie (pers. comm.), working on soils in the area, has found that the following concentrations of nutrients are present in the soil solution. (Results have been corrected to a soil moisture content of 20%).

Soil type	P	K	Mg
	p.p.m.	p.p.m.	p.p.m.
Kaingaroa (strongly leached)	0.015	17	1.7
Taupo (moderately leached)	0.033	21	7.3

These figures suggest that the concentration of P in the actual soil solution is of a low order in many forests where tree growth is good. Certainly concentrations will never approach the constant level of 300 p.p.m. P which Mitchell (1939) and Travers (1959) suggest is needed for optimum growth. It seems, then, that these studies have been carried out under overall nutrient levels which are much higher than those found under field conditions in most places.

The levels of nutrients in the foliage of seedlings do seem to offer a more practical means of determining whether supplies are sufficient to maintain optimum growth. Boszormenyi (1958) has shown that critical foliage levels of N and P respectively in the foliage of Scots pine each increase about threefold when supplies of the other are raised from very low to very high. However, such variations in the supply of nutrients are not likely to be found between one forest soil and another. The rates at which nutrients were supplied in this present series of experiments do seem to approximate to field conditions because the critical foliage levels found for all nutrients agree with those values that are available from cases of known deficiencies in nurseries and forests.

Included in Tables 1–7 are the weights, in the shoots and roots, of the nutrient that was varied. The amount of a nutrient in the shoots was consistently greater than in the roots. Also the amount in both the shoots and roots increased with increasing supply, except for N and K in the roots when they were supplied at high rates. Under deficient conditions, the amounts of N and Mg taken up by seedlings were relatively constant and did not vary with the supply.

Brown *et al.* (1959) have discussed the occurrence of chlorosis in plants, and its causes, one of which is the decrease in Fe uptake under conditions of high P. Low supplies of N and K have resulted in high concentrations of P in seedlings and to some extent lower uptakes of Fe. In N-deficient seedlings the induced chlorosis was severe, masking any other effects, but it was possible to reduce the chlorosis in K-deficient seedlings by supplying Fe in the form of Fe—EDTA. It seems, then, that K deficiency can be associated with an Fe deficiency owing to a high P uptake, and both contribute to the chlorosis.

It must be pointed out that not all cases of chlorosis are due to a mineral deficiency in the soil. Fungal attack (on roots or shoots), insect damage, the chewing of bark by browsing animals, and anaerobic soil conditions, can all result in needle chlorosis. Gerhold (1959) has shown that the varying degree of winter chlorosis in different races of Scots pine is unrelated to mineral nutrition. The Genetics Section of the Forest Research Institute, Rotorua, has under observation a few clones of radiata pine that have remained chlorotic even after grafting on to other root stock.

Work is being carried out on Ca deficiency but, because this nutrient accumulates in ageing tissue, it may not be possible to arrive at a narrow range of values indicating deficiency.

Supplies of Na have not been progressively increased in a series of experiments, but a wide range of concentrations of Na in shoots and roots resulted from variations in the supply of other nutrients. As all solutions were made from reagent grade salts, those that did not include an Na salt would not have had any great concentration of Na. Yet, in some of these cases (e.g., high supplies of K and Mg), very high concentrations of Na were found in plants. It seems that under some circumstances the plant will selectively take up Na.

Conclusions

1. P deficiency results in blue-green foliage, but N, K, and Mg deficiencies all produce chlorosis. The type of chlorosis is different for each deficiency: N chlorosis is even over the whole plant, K chlorosis is more pronounced in the needle tips, while Mg chlorosis is restricted (except in very severe cases) to the tips of needles and is much more golden in appearance.
2. N and P deficiencies are characterised by increased root growth.
3. The following concentrations of nutrients in water-culture solution are needed to maintain good growth under the conditions of the experiment. At lower concentrations growth rates are reduced and deficiency symptoms occur.

   N—About 100 p.p.m., certainly more than 25 p.p.m.

   P—1 p.p.m.

   K—Probably 5–10 p.p.m. but possibly more.

   Mg—Between 3 p.p.m. and 25 p.p.m. Probably about 10 p.p.m.

   In perlite culture older plants need a greater supply of K and probably of P.
4. The ratios of the concentration of a given nutrient to the concentration of other nutrients, within limits, have more influence on plant growth than have the actual concentrations. This is shown by the rise in the optimum level of supply of any one nutrient with the concentration of other nutrients. It is essential, therefore, to carry out tree nutrition experiments using a basic formula in which the nutrient concentrations are

of the same order as those found in soil solutions in forest regions. The present series of experiments appears to have met this condition.

5. The following are critical levels of nutrients in the foliage below which growth is restricted:

   N—About 1.6%, provided no chlorosis is present.

   P—About 0.10%.

   K—1.1%–0.7%.

   Mg—0.11%–0.08%, provided no chlorosis is present.

6. A low level of N, P, or K in the foliage indicates a deficiency of that element, but high levels can be caused by: (i) excess supply of the element concerned; or (ii) the deficiency of another nutrient.

7. N and K are fairly evenly distributed between the shoots and roots, but concentrations of P and Mg tend to be higher in the roots. Under deficiency conditions the level of P in the roots tends to be maintained at the expense of the shoots. In the case of N deficiency, levels of shoot N are maintained at the expense of the roots.

8. Almost all the Fe in a seedling is in the roots.

9. Roots show changes in composition that do not affect the shoot, e.g., low and high Mn levels with low P and high N supplies respectively.

10. Concentrations of Na in a plant vary markedly with the supply of Na and with deficient or excess supplies of other nutrients.

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OESTROGENS IN PASTURE LEGUMES

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Summary

The oestrogenic activities of a number of varieties of white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), and lotus (*Lotus uliginosus* L.) were tested by incorporating the freeze-dried plant material in the food of ovariectomised immature mice. Oestrogenic activity was detected in all the samples of red clover but not in the white clover or lotus samples.

Introduction

Many types of plants have been found to contain substances which have oestrogenic activity or which give rise to other substances with oestrogenic activity when consumed by animals. The older studies of such plants and substances have been reviewed by Bradbury and White (1954) and Pope (1954), while Biggers (1959) has written a general review of these older and some later studies. Recently additional evidence of oestrogens in plant species commonly eaten by grazing animals has been given by Fox and his colleagues in Oregon (Fox, Kaufmes, *et al.* (1958); Fox and Oldfield (1958); Fox, Mason, *et al.* (1958); Kitts, *et al.* (1959a, b) in British Columbia; and Pope, *et al.* (1959) in England. Bickoff *et al.* (1957) reported the interesting finding in ladino clover (*Trifolium repens* L.) of coumestrol which has considerably greater oestrogenic activity than the oestrogenic isoflavones known in subterranean clover (*Trifolium subterraneum* L.) and red clover (*Trifolium pratense* L.).

In New Zealand, Cunningham and Hogan (1954) found oestrogenic activity in subterranean clover and red clover and a suggestion of slight activity in some samples of perennial ryegrass (*Lolium perenne* L.). Bassett and White (1955) tested a large number of plant species

common in dairy pastures, and obtained positive responses from subterranean clover, red clover, and one of several samples of suckling clover (*Trifolium dubium* L.). Ch'ang (1958) at Massey College found that ewe lambs which had been grazing a pasture containing red clover showed mating behaviour without subsequent formation of corpora lutea in the ovaries, and red clover from this pasture was found to be oestrogenic.

Because oestrogenic activity seemed to be widespread in species of pasture plants, and in the study made by Ch'ang the activity of red clover had apparently been sufficiently high to affect the reproductive activity of grazing sheep, it was decided to subject to biological test with mice a number of varieties of different species of legumes. Subterranean clover which has been clearly shown to reach levels of activity sufficient to affect sheep severely in Australia (see reviews cited earlier) was not included in the study.

Materials and Methods

Plant Material

Ten or 20 plants of each of a number of varieties of white clover (*Trifolium repens* L.), red clover (*T. pratense* L.), and (*Lotus uliginosus* L.) were grown at Grasslands Division, D.S.I.R., Palmerston North, in a replicated plot system, and some additional red clover was obtained from swards at Massey College. The green material, comprising leaf, petioles, and stems in about the same proportion as in the growing plants, was harvested on 6 January and 26 March 1959. The green material was placed in plastic bags, which were in turn put in large tins with tight-fitting lids. These tins were taken within three hours to a cool room maintained at a temperature below —15°c. When required for testing, the plant material was freeze-dried, ground finely in a Wiley mill, and added to meal mixture consisting of two parts of barley meal to one of dried buttermilk powder, so that the plant powder made up 20% by weight of the final mixture.

Mice

The mice were from an albino strain inbred to an unknown extent but recently maintained by random breeding. Females were ovariectomised at 21 or 22 days of age and started on experimental diets one or two days later. Those under 8.5 g body weight were rejected because small mice sometimes do not do well on diets containing this amount of plant material. Mice being fed on the same diet were housed together, not more than six to a box, with a permanent water supply, and the temperature was maintained at approximately 21°c. The rate of feeding was 2.5 g of dry diet per mouse per day, any food remaining from the previous day's feed being weighed and recorded. Control mice were fed a similar diet containing plant material known to be oestrogenic. Feeding on the test diet continued for 10 days. The mice were slaughtered on the eleventh day, and the uteri were blotted and weighed immediately on a torsion balance.

Text continued on page 332

TABLE 1. Mean Uterine Weights of Mice Fed Plant Material

Species	Variety	Date of Harvest (1959)	Experiment No.	Storage Age (weeks)	Mean Uterus Weight (mg)		Level of Significance of Difference (Duncan's Range Test)†
					Treated	Controls	
Trifolium repens	High HCN (N.Z. selection)	6/1	3	22	5.9*	5.6	> 0.1
			7	30	4.8	4.6	,,
		26/3	6	18	4.5	4.3	,,
			7	19	4.9	4.6	,,
	Glucoside-free (N.Z. selection)	6/1	2	18	6.3	5.9	,,
			3	22	5.7	5.6	,,
		26/3	6	19	5.5*	4.3	> 0.05 < 0.1
	(ex Spain)	6/1	3	22	5.9	5.6	> 0.1
			4	26	4.4*	4.9	,,
	Ladino, (ex California)	6/1	2	18	5.7	5.9	,,
			3	22	6.2	5.6	,,
		26/3	7	19	4.1	4.6	,,
Lotus uliginosus	Diploid (ex Portugal)	6/1	3	22	4.6	5.6	,,
			4	26	4.4	4.9	,,
		26/3	5	17	4.4	5.1	,,
	Diploid (N.Z. Selection)	6/1	2	18	7.2	5.9	,,
		26/3	4	26	5.5	4.9	,,
			6	19	5.0	4.3	,,
	Tetraploid (N.Z. Selection)	6/1	1	16	5.9*	6.0	,,
			5	28	4.3	5.1	,,
Lotus corniculatus	(ex Oregon)	6/1	1	16	5.2	6.0	,,
			3	22	6.0	5.6	,,
Lotus tenuifolius	(probably ex U.S.A.)	6/1	4	26	4.9	4.9	,,
		26/3	5	17	5.8	5.1	,,

Trifolium pratense	Montgomery (a)	6/1	1	16	13.8	6.0	< 0.001
	(N.Z. Certified)	6/1	2	18	15.6	5.9	,,
	(b)	26/3	6	18	9.5	4.3	,,
	Montgomery	6/1	2	18	18.2	5.9	
	(N.Z. Certified)	6/1	5	28	9.7	5.1	,,
	(Sward)	26/3	6	18	7.6	4.3	,,
	S123 Montgomery (a)	6/1	4	26	11.0	4.9	< 0.001
		26/3	5	17	8.3	5.1	< 0.01
	(b)	6/1	2	18	7.8	5.9	< 0.20
		26/3	5	17	11.6	5.1	< 0.001
	Broad	6/1	3	22	16.4	5.6	,,
	(N.Z. Certified Cowgrass)	26/3	5	17	8.1	5.1	< 0.01
	(b)	6/1	7	30	10.5	4.3	< 0.001
		26/3	7	19	6.9	4.3	< 0.05
	Broad	6/1	2	18	10.0	5.9	< 0.01
	(N.Z. Certified Cowgrass: Sward)	6/1	5	28	7.1	5.1	< 0.05

Footnote: 6 mice per group except for those marked *, where two replicates were combined, giving 12 mice per group.

Data were transformed to logarithms for analysis; no standard errors are presented.

(a) & (b). In some cases two replicate groups of plants of the same species and variety were grown separately and tested separately.

Results for such replicates are shown by the letters (a) and (b).

† Duncan, D. B. 1955: *Biometrics 11:* 1.

The tests were made in a series of experiments, each including its own group of control mice and material from different species and varieties of plants. Six mice, allotted at random, were used for each treatment. Because mice become available over a period of time the groups were filled gradually.

Results

The mean uterine weights for the groups of treated and control mice are shown in Table 1. All the red clover samples caused vaginal opening in all the mice to which they were fed. No mice fed the other plant materials showed vaginal opening.

The only species clearly shown to contain material with oestrogenic activity, or material which gave rise to oestrogenic activity, as judged by increases in uterus weight, was red clover (*T. pratense*). Mice in two groups fed cyanogenetic-glucoside-free white clover in the same experiment had uterus weights which exceeded those of the controls in the same experiment by 1.2 mg, this difference being significant at the 10% level. If this difference was due to oestrogenic activity arising from the ingestion of clover it appears that the level of activity was very low in comparison with that of the red clover samples tested.

Two samples of red clover (Montgomery, Sward) taken at the same harvest date were tested twice, after different periods of storage, and in each case caused smaller increases in uterine weight after the longer storage period. The possibility of changes in sensitivity of the mice to oestrogen with time made tests of the significance of the differences between samples tested in different experiments of little value.

Discussion

In two respects the technique was not fully satisfactory. Because some of the samples were discoloured, as though "sweating" had occurred, it seems that some of the plant material was not frozen initially as quickly as was desired, probably because of the insulating effect of the tins filled with plastic bags. In addition, the mice were able to do some sorting of the meal mixture. Because the amount fed was restricted this did not result in much being left, but it seemed that some mice had more and some less of the oestrogenic material than others. In work done later than that reported here, when wholemeal flour replaced barley meal in the mixture, the variation between uterine weights of mice in the same treatment group was reduced (Munford and Flux 1961).

In contrast with the experience of Bickoff *et al.* (1959) with ladino clover (*T. repens* L.), it was possible to incorporate 20% of dried plant material in the ration and, in the case of red clover, to obtain large responses in uterine weight increases. In a later experiment, in which leaves and soft stems of a lotus species grown in a glass-house were freeze-dried and mixed with a similar meal mixture, the food intakes of the mice were poor, and they did not maintain body weight (Flux and Munford unpublished data). This plant material

was between three and four times as bulky as the same weight of red clover, hence it appears that density may be important in determining how much dried plant material can be incorporated in a ration.

The indication of decline in activity with storage in a deep freeze given by the two tests of the samples of Montgomery and broad red clover was checked by a retest of another sample of Montgomery red clover approximately a year after it was first tested (Ch'ang 1958). This sample, which had been stored in a deep-freeze, no longer caused a significant increase in the mean uterus weight of the mice to which it was fed (6.73 ± 0.36 mg for uteri of six control mice, and 6.00 ± 0.36 mg for those of six treated mice). Because it is possible that similar declines in activity occurred during storage of the other clover samples tested, the results reported in Table 1 should be regarded as likely to show less oestrogenic activity than that which existed in the dry material of the freshly harvested plants. As far as the present study was concerned this would not be of great importance provided that sufficient activity remained in the plants which did contain oestrogenic material for them to be rated as positive. However, it would be important if quantitative comparisons were to be made.

Even comparisons of the relative activity of different samples of materials tested at the same interval after harvesting could be made only if it could be assumed that the rate of decline of activity was the same for all samples. It is doubtful whether this could be safely assumed because red clovers have been reported to contain a number of isoflavones of very similar chemical structure genistein, genistein 4'–methyl ether (biochanin A) (Pope *et al.* 1953; Pope 1954), possibly a water soluble conjugate of one of these (Pope *et al.* 1959), and the inactive or only slightly active formononetin (Bate-Smith *et al.* 1953), as well as coumestrol (Bickoff *et al.* 1957). Declines with drying in the activities of subterranean clover (Alexander and Watson 1951) and of ladino clover, in which the most important oestrogen was coumestrol (Bickoff *et al.* 1959), have been reported; but, as far as the present authors are aware, there have been no other reports on the stability of oestrogenic activity after freeze-drying.

The main measure of activity used in these experiments, increase in the uterine weight of ovariectomised mice, has been criticised by some authors (such as Biggers 1959) because it is not specific, and these workers prefer to use instead cornification of the vaginal epithelium. However, all oestrogens do not have the same dose response pattern; for instance, in the mice from the same colony as those used in the present study, diethylstilboestrol in doses causing uterine weight increases of the same order as did many red clover samples, had little or no effect on vaginas (Munford and Flux 1961), hence uterine weight increase seemed a better endpoint to use in initial screening tests. The later work showed that red clover samples did cause vaginal cornification.

Bickoff *et al.* (1959) suggested the use of immature intact mice because they were more sensitive than ovariectomised mice. In the comparison shown by these authors the ovariectomised mice

were older and larger than the intact immature mice. Ovariectomised mice of the strain used in the present study showed statistically significant increases in uterus weight when given total doses of less than 0.03 μg diethylstilboestrol, and, judging by the dose needed to produce a tripling of uterus weights, were of the same order of sensitivity as the immature mice of Bickoff *et al.* (*loc. cit.*).

The recent finding of Kitts *et al.* (1959b) that alfalfa, ladino clover, and birdsfoot trefoil, although free from oestrogenic activity in their first year, were active in the second year of growth is important in relation to the present results, which were all obtained from plants in their first year of growth. The ladino clover used should at least be tested in the second year because of the finding of coumestrol in the species by Bickoff and his colleagues (*loc. cit.*).

Red clovers seem to have been found to be oestrogenic by all who have tested them, but there is not a great deal of conclusive evidence that they have adversely affected livestock. Fox and Oldfield (1958) in Oregon reported a lower lambing percentage and a more extended lambing season for ewes grazing red clover pasture than for others fed red clover hay or alfalfa hay, but the numbers of animals were too small to exclude chance as a likely cause of the differences. In New Zealand, Cunningham and Hogan (1954) reported evidence of oestrogenic effects in wethers grazing pasture in which red clover was predominant but which also contained subterranean clover. Although the latter had passed its peak growth period it is not clear whether it or other species of plants contributed oestrogen to the wethers, and no tests of oestrogenic activity of individual plant species were made. Ch'ang (1958), as previously mentioned, found effects of oestrogen in ewe lambs grazing pasture containing red clover shown to be oestrogenic. Ch'ang (pers. comm.) also found considerable mammary development, including a milk-like fluid in the mammary glands of unmated mature ewes grazing a similar pasture. There have been no published reports of effects on sheep similar to these of subterranean clover in Australia (see reviews cited earlier). However, it would probably be wise in the future to take notice of the oestrogenic activity of red clovers. Pastures containing active varieties of this species may be useful for fattening stock but might be dangerous for breeding animals, particularly sheep, which, in New Zealand, are approaching, or are in their mating season when red clover is making its most vigorous growth. Most cows are in calf by this time of the year, and it is not expected that they would be affected.

At present little appears to be known of the importance to the plant of isoflavones, which include those compounds which are major contributors to oestrogenic activity in red clover. Investigation of the effect of changes in the environment of red clover on oestrogenic activity might be important. Alexander and Rossiter (1952) suggest that in the case of subterranean clover oestrogenic activity was related to soil phosphate levels, and it may be that

red clover can be used more safely in some soils and climates than in others.

A main object of the present study was to test a number of varieties of lotus, red clover, and white clover species, these being of particular interest to plant breeders in New Zealand. Despite the difficulties encountered with storage of material and feeding of mice in the early stages of the work, the tests were quite adequate to show that all the red clover varieties contained appreciable quantities of oestrogenic material, while the lotus and white clover varieties did not.

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NEW RECORDS OF FUNGOUS DISEASES IN NEW ZEALAND, 1960–1

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Summary

The following fungi are recorded from New Zealand for the first time:—*Elsinoe mattirolianum* (Sacc. & D. Sacc.) Arnaud & Bitancourt, *Nectria haematococca* Berk. & Br. var. *cucurbitae* (Snyder & Hansen) n. comb., *Sclerotinia squamosa* (Viennot-Bourgin) Dennis, *Stagonospora hortensis* Sacc. & Malb., *Colletotrichum dematium* (Berk.) Vogl. var. *circinans* (Berk.) Arx, *Alternaria cucumerina* (Ell. & Everh.) Eliot, *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen var. *cepa* (Hanz.) Snyder & Hansen.

Additional host records include *Albugo tragopogonis* Pers. ex. S. F. Gray on *Senecio vulgaris* L.; *Taphrina cerasi* (Fckl.) Sadb. on *Prunus yedoensis* Matsum; *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & de Not. on *Rosa rubiginosa* L.; *Fabraea maculata* Atk. on *Crataegus monogyna* Jacq.; *Corticium rolfsii* (Sacc.) Curzi on carrot (*Daucus carota* L.), artichoke (*Helianthus tuberosa* L.), and kumara (*Ipomoea batatas* Poir.); *Corticium salmonicolor* Berk. & Br. on pear (*Pyrus communis* L.); *Septoria cucurbitacearum* Sacc. on cucumber (*Cucumis sativa* L.) and on rock melon (*Cucumis melo* L. var. *cantalupensis* Naud.); *Botrytis gladiolorum* Timmerm. on *Freesia refracta* Klatt; *Fusicladium carpophilum* (Thum.) Oudem. on almond (*Prunus amygdalus* Batsch.).

The following records are based on naturally infected material collected and identified during 1960–1. In all cases the specimens have been placed in the herbarium of the Plant Diseases Division, Department of Scientific and Industrial Research, Auckland. The herbarium number is given in brackets after the locality of the record.

1. ELSINOE MATTIROLIANUM (Sacc. & D. Sacc.) Arnaud & Bitancourt, Mycologia, *41*, 322, 1949.

on leaves of *Arbutus* sp. Gore, Southland, December 1959, ?, (19121).

Lesions 2–5 cm. diam., epiphyllous, circular, often coalescing, reddish brown with darker margins, becoming greyish white in centre; ascomata solitary, sometimes fusing in centre of lesion, formed immediately beneath the cuticle of

the leaf; when young, i.e., when ascomata are about 30μ diam, conidiophores form a superficial layer on the developing stroma, rupture cuticle and bud off conidia. Conidia lenshaped, up to 5μ long, non-septate. Mature ascomata pseudoparenchymatous, up to 0.1 mm. diam.; asci globose, 10–20 × 8.5–12μ formed within the tissue, eight spored. Ascospores one septate, hyaline, 8–10 × 4–5μ.

Jenkins (1933) examined material of *Illosporium mattiroli-anum* Sacc. & D. Sacc. and showed that this fungus was typical of the genus *Sphaceloma*. Later Arnaud and Bitancourt (1949) examined herbarium material of this fungus and found a collection containing asci and spores; they then placed the species in *Elsinoe*. In the New Zealand collection asci are smaller and more globose than those described by these authors.

2. NECTRIA HAEMATOCOCCA Berk. & Br. var. CUCURBITAE (Snyder & Hansen) n. comb.

Syn. *Hypomyces solani* Rke. & Berth. emend. Snyder & Hansen var. *cucurbitae* Snyder & Hansen Amer. J. Bot. *28*, 741, 1941.

Conidial form *Fusarium solani* var. *cucurbitae* Snyder & Hansen.

Syn. F. *javanicum* Koord.

on roots and stems causing a basal rot of pumpkin (*Cucurbita pepo* L.). Kumeu, Auckland, January 1960. B. Coleman (19146).

and on melon (*Citrullus vulgaris* L.) Kumeu, Auckland, January 1960 J.M.D. (19147).

Lesions formed at stem base of seedlings or on fruits, as discreet, light coloured lesions up to 1 mm. diam., often coalescing. Sporodochia up to 0.5 mm. diam., orange, slimy. Macroconidia falcate, slightly curved, often only at apex, 1–5 septate, usually 3; 1 septate 10–17.5 × 4.5–5μ, 3 septate 25–48 × 4–5μ, 5 septate 40–65 × 4–5μ. Microconidia unicellular, sometimes one septate, 8–15 × 3–3.5μ.

Perithecial form—no fertile perithecia have been collected in New Zealand.

On potato dextrose agar, macroconidia are freely produced on orange sporodochia. Cultures are quick growing, floccose, isabelline with some greenish blue pigmentation. Doidge and Kresfelder (1932) first recorded this fungus as *Fusarium javanicum* Koord., the causal organism of root rot of squash in South Africa. Later Snyder (1938) recorded the same disease from North America, and Conroy (1953) from Australia.

Snyder & Hansen (1941) stated that *F. javanicum* Koord. is a synonym of *F. solani* (Mart.) Appel & Wollenw. emend. Snyder & Hansen and gave this pathogenic species the name *F. solani*

var. *cucurbitae*, listing the perithecial form as *Hypomyces solani* var. *cucurbitae*. Booth (1960) showed that *Hypomyces solani* Rke. & Berth. is not associated with the conidial form *F. solani* and therefore cannot be considered a synonym of *Hypomyces haematococca* (Berk. & Br.) Wollenw. as stated by Snyder & Hansen (1941). Furthermore, as *H. haematococca* is not typical of *Hypomyces*, a genus restricted to only species with one septate fusiform or elliptical, apiculate ascospores as defined by the type species *Hypomyces lactifluorum* Schw., it is necessary to recognise the perithecial form of *Fusarium solani* as *Nectria haematococca*. As this fungus is morphologically identical with *N. haematococca* but restricted in its pathogencity to species in the Cucurbitaceae it is recognised as a variety.

The fungus is not uncommon in the Auckland area, especially where pumpkins have been grown for a number of years. Conroy (1953), and Tousson and Snyder (1961) both report that the disease, as well as being soil borne, is seed carried, mycelium being present in the outer seed coat. Tousson and Snyder (1961) report that none of the varieties

Fig. 1.—*Sclerotinia squamosa* causing tip dieback lesion on onion seedlings showing conidiophores. (Photo—S. A. Rumsey.)

Fig. 2.—White flecks on leaf blades due to infection of *Sclerotinia squamosa*. (Photo—S. A. Rumsey.)

tested in the laboratory proved to be resistant, although some varieties appear to have field resistance; they also state that a cucurbit free rotation of four years is generally sufficient to reduce the disease to a level at which it is not a problem to the grower.

3. SCLEROTINIA SQUAMOSA (Viennot-Bourgin) Dennis, Mycol. Papers, Kew, *62*, 157, 1956.

Conidial form *Botrytis squamosa* Walker

on seedling onion plants (*Allium cepa* L.) Pukekohe, Auckland, July 1960 K.M. Harrow (19482) (19508).

Lesions formed at apex of leaves causing tip dieback, or as minute white flecks 1 mm. long on leaf blades. Conidiophores numerous on tip dieback lesions only, produced singly, with a distinct basal cell, septate and pigmented brown, 100–200 × 17.5–20μ sparsely branched at apex. Conidia budded off from short apical branches, hyaline, oval or globose 11–27 ×

11.5–24μ, apex of spent conidophores collapsed in characteristic accordionlike folds.

No apothecia have been collected in New Zealand.

Walker (1925) differentiated this species from *B. allii* Munn by its larger spores and characteristic method of collapse of the spent conidiophores. Conidial measurements of New Zealand material agree with those given by Dennis (1956) while conidia from cultures are smaller but typical of the measurements given by Walker (1925).

This fungus was widespread in the onion crops in the Pukekohe district, causing considerable damage, especially at the seedling stage, during the spring of 1960 (Fig. 1, 2.).

4. STAGONOSPORA HORTENSIS Sacc. & Malb. Michelia *2*, 629, 1882.

Syn. *Ascochyta boltshauseri* Sacc. Z. Pflkrankh. *1*, 136, 1891.

on leaves and petioles of beans (*Phaseolus vulgaris* L.) Remuera, Auckland, June, 1960, J.M.D. (19321).

Lesions numerous, light fawn, up to 1 cm. diam., circular, usually zonate, often fusing. Pycnidia numerous, immersed in upper surface of leaf, arranged in concentric rings in lesion, ostioles rupturing epidermis. Pycnidia globose, 115–160μ diam., 2–3 cells thick, lightly pigmented. Conidia hyaline, cylindrical with rounded ends or oval, 1–3 septate, 21–34 × 3–8μ.

Sprague (1935) recorded *Ascochyta boltshauseri* from North America, Europe and New Zealand. There is a single record of this fungus in New Zealand on broad bean (*Vicia faba* L.) by Kirk (1906).

Unfortunately no herbarium material of this record can be traced but as the fungus has been shown to be host specific by Sprague (1935) it is presumed that Kirks' record is a misdetermination of *Ascochyta fabae* Speg.. The present collection of *Stagonospora hortensis* is from a late crop of rust resistant beans (var. Westralia) in a home garden. Lesions were present on leaves and petioles causing premature defoliation of the plants.

Most conidia were 2–3 septate, suggesting that the fungus was more typical of *Stagonospora* than *Ascochyta*. Petrak (1921) stated that *Ascochyta boltshauseri* is a synonym of *S. hortensis* and as spores vary from 1–3 septate he placed it in Diedicke's genus *Stagonosporopsis*. In 1925 Petrak followed the revision of the Sphaeropsidales by von Hohnel and reduced *Stagonosporopsis* to a synonym of *Ascochyta*. Sprague (1935) therefore used the name *Ascochyta boltshauseri* for this species stating that he hesitated to follow Petrak's lead in adopting the name *Stagonospora hortensis*, for he doubted if Petrak had examined type material. He preferred to recognise the genus *Ascochyta* to

include species which at times form 2–3 septate spores and to use the name *A. boltshauseri.*

Petrak (1943) re-examined *Ascochyta boltshauseri* and *Stagonospora hortensis* and stated that the species are synonymous and typical of the genus *Stagonospora.* The specific epithet '*hortensis*' thus has priority over '*boltshauseri*'.

5. COLLETOTRICHUM DEMATIUM (Berk.) Vogl. var. CIRCINANS (Berk.) Arx Phytopath. Z., *29,* 461, 1957.

Syn. *C. circinans* (Berk.) Vogl., Ann. Acad, Agric. Torino, 49, 175, 1907.

on seedling onion plants (*Allium cepa* L.) Pukekohe, Auckland, December 1959, K. M. Harrow (19483).

Lesions poorly defined on leaf blades and petioles of young plants or as a sooty blotch on mature bulbs. Acervuli superficial formed on minute dark coloured pseudoparenchymatous stromata, 0.1–0.5 mm. diam., sunken beneath the cuticle. Setae 112–250 × 5μ, formed among the hyaline conidiophores and projecting above the fructification. Conidia unicellular, hyaline fusiform, often falcate, 16–25 × 1.5– 4μ, catenulated from apex of conidiophores.

Walker (1920) recorded *C. circinans* as a weak pathogen of onions, causing smudge of stored onion bulbs, especially the white skinned varieties. The variety 'Pukekohe Long Keeper' commonly grown in the Auckland area appears to be moderately susceptible

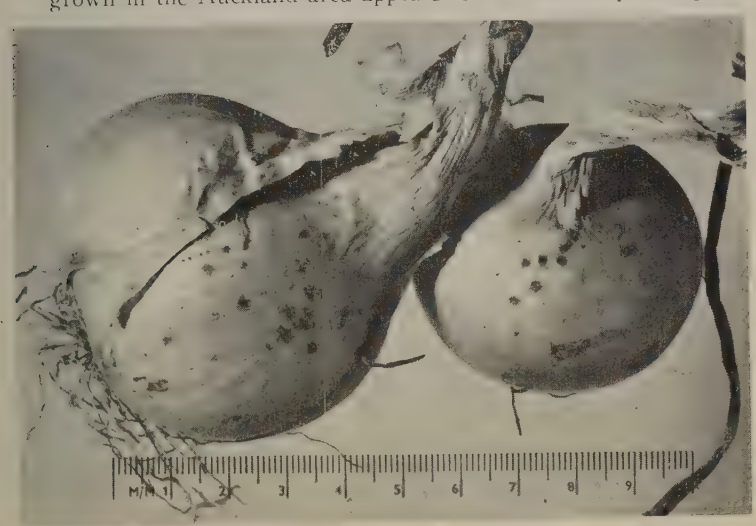

Fig. 3. Sooty blotch due to *Colletotrichum dematium* var. *circinans* on onion bulbs (var. Pukekohe long keeper). (Photo—S. A. Rumsey.)

to the disease (Fig. 3). In New Zealand this fungus also causes a damping off of young seedlings. Brooks (1953) stated that infection comes from the soil and may occasionally cause a damping off. The fungus has been recorded from Europe, North America and Australia.

6. ALTERNARIA CUCUMERINA (Ell. & Everh.) Eliot, Amer. J. Bot., *4*, 439, 1917.

Syn. *Macrosporium cucumerinum* Ell. & Everh. Proc. Acad. nat. Sci. Philad. 440, 1890.

Sporidesmium mucosum Sacc. var. *pluriseptatum* Karst. & Har., J. of Bot., Paris, *4*, 363, 1890.

S. pluriseptatum (Karst. & Har.) Peck, Bull N.Y. St. Mus. *131*, 27, 1909.

on leaves of *Cucumis sativa* L., Mangere, Auckland, November 1959, J. White (19132).

Lesions on leaves, and stems, circular often constricted at veins, up to 2 cm. diam., sometimes zonate, brown, light coloured when old. Dark coloured conidia often present on under surface of leaf. Conidiophores formed in fascicles of up to 6, septate, pigmented, 21–75 × 4–6μ. Conidia usually borne singly, sometimes in small chains, usually at apex of conidiophore; pigmented obclavate, ovate or broadly ellipitical, muriform, with 3–8 transverse septa 19.5–72 × 9.5–13μ, sometimes with a septate beak up to 24μ long.

Spore suspensions from cultures prepared from this material inoculated on to cucumber plants produced similar lesions within a week of inoculation. Check plants remained uninfected. At present the fungus has been found only in a single glass-house crop of cucumbers in Auckland where it has appeared for two successive seasons.

Examination of naturally infected material and cultures showed this species to be highly variable. Conidia measurements are not as large as those given by Jackson and Weber (1959) but agree more closely with those cited by Saccardo and Sydow (1899). The description of *Sporidesmium pluriseptatum* given by Neergaard (1945) suggests that this species is synonymous with *Alternaria cucumerina*. He recorded it is a pathogen on cucumbers and melons in Denmark, Norway and Germany. It has been recorded from North and South America, Sudan, South Africa and Australia.

7. FUSARIUM OXYSPORUM Schlecht. emend. Snyder & Hansen var. CEPA (Hanz.), Snyder & Hansen, Amer. J. Bot., *27*, 66, 1940.

on onion plants (*Allium cepa* L.) causing a basal rot. Pukekohe, Auckland, December 1959, K. M. Harrow (19154).

Lesions formed at the base of the bulb destroying a large number of rootlets and basal portion of bulb scales, characteristically only on one side of bulb. Pigmented, salmon to carmine, mycelium present among scales; slimy sporodochia 1–2 mm. diam. often present among mycelium. Macroconidia arcuate, 1–3 septate, hyaline, 16–48 × 3–4μ.

On potato dextrose agar at 24°c, the fungus forms moderately quick growing salmon coloured colonies darkening with age; sporodochia 0.1–0.5 mm. diam. slimy, cream or salmon coloured; macroconidia 16–32 × 2.5–3.5μ, microconidia very common, elliptical often allantoid 8 × 1.5–2μ; terminal and intercalary chlamydospores often present, 10–15μ diameter.

F. oxysporum var. cepa is described from North America (Walker & Tims 1924) and South Africa (du Plessis 1933). It is also recorded from Japan, South America, the Philippine Islands and Australia. In New Zealand basal root rot of onions is not uncommon in the Pukekohe area and specimens have been

Fig. 4.—Onion bulbs infected with *F. oxysporum* var. *cepa* showing the characteristic one-sided infection. (Photo—J. W. Endt.)

collected from Katikati, Bay of Plenty. The characteristic one-sided infection of the growing bulbs led to the local name of "leaners" (Fig. 4).

Additional Host Records

8. ALBUGO TRAGOPOGONIS Pers. ex S. F. Gray, A Natural Arrangement of British Plants, *2*, 540, 1821.

on leaves of *Senecio vulgaris* L. Palmerston North, Wellington, June 1960, G. N. Paulin (19704).

9. TAPHRINA CERASI (Fckl.) Sadb., Jb. Hamburg. Wiss. Anst., 8, 61, 1891;

Syn. *T. minor* Sadb., Jb. Hamburg. Wiss. Anst., *8*, 61, 1891.

on leaves of *Prunus yedoensis* Matsum, Botanic Garden, Wellington, October 1960, R. A. Campion (19509).

10. BOTRYOSPHAERIA DOTHIDEA Moug. ex Fr.) Ces. & de Not. Comment Soc. Crittogam Ital. *1*, 212, 1863.

on stems of *Rosa rubiginosa* L. Wairau Valley, Marlborough, June 1960, J. P. Begg (19310).

Previously this fungus has been recorded from New Zealand as *Botryosphaeria ribis* Gross. & Dugg. (Cunningham 1922). Von Arx and Mueller (1954) showed that this species is a synonym of *B. dothidea*, the type of this species being on *Rosa rubiginosa*.

11. FABRAEA MACULATA Atk. Science *3*, 452, 1909.

on fruits of *Crataegus monogyna* Jacq. Pirongia, Auckland, February 1960, J.M.D. (19796).

Only conidia were present on disease lesions on hawthorn fruits. In the hedge row in which this specimen was collected most of the fruits were infected. *F. maculata* was first recorded from New Zealand on quince (*Cydonia oblonga* Mill.) and pear (*Pyrus communis* L.) by Kirk (1894).

12. CORTICIUM ROLFSII (Sacc.) Curzi Boll. Staz. Pat. veg. Roma, n.s. *11*, 365, 1932.

on roots of carrot (*Daucus carota* L.) Te Atatu, Auckland, March 1960, B. Coleman (19182).

on tubers of artichoke (*Helianthus tuberosa* L.) Mt. Albert, Auckland, March 1961(?) (19834).

on tubers of kumara (*Ipomoea batatas* Poir.) Avondale, Auckland, November 1960, B. Coleman (19715).

C. rolfsii has recently become very common in the Auckland district, attacking a wide range of plants, especially summer crops and bulbs.

13. CORTICIUM SALMONICOLOR Berk. & Br. J. Linn. Soc. (Bot.) *16*, 71, 1873.

on stems of pear (*Pyrus communis* L.) Kerikeri, Auckland, November 1960, L. Saxton (19643).

This is the second recording of this common tropical pathogen in New Zealand. The first record was from a deserted orchard (Brien & Dingley 1957). Now it has become common in this local area near Whangarei, but only on apple trees (*Malus sylvestris* Mill.). Again it appeared on an isolated tree, this time in a home garden.

14. SEPTORIA CUCURBITACEARUM Sacc. Nuovo Giorn. Bot. Ital. *8*, 205, 1876.

on leaves of apple cucumber (*Cucumis sativa* L.), Avondale, Auckland, January 1961, J. Blacker (19703)

on leaves of rock melon (*Cucumis melon.* var. *cantalupensis* Naud.), Auckland, February 1959, J. White (18636).

Septoria leaf spot of cucurbits is not uncommon in the Auckland area.

15. BOTRYTIS GLADIOLORUM Timmerm, Meded Lab. Bloembollenond Lisse *67*, 15, 1941.

on *Freesia refracta* Klatt Oratia, Auckland, August 1960, J. Watkins (19479).

B. gladiolorum is common on *Gladiolus* spp. as a corm rot and on leaves and flowers. Re-examination of herbarium material of previous records of *Botrytis cinerea* (Pers.) Fr. on gladiolus (Brien and Dingley 1953) has shown it to be typical of *B. gladiolorum.*

16. FUSICLADIUM CARPOPHILUM (Thum.) Oudem. Kon. Akad. Wet. Amst., 394, 1900.

Syn. *Cladosporium carpophilum* Thum. Oest. Bot. Z., 27, 12, 1877.

on almond fruits (*Prunus amygdalus* Batsch.) Motueka, Nelson, March 1961, G. F. Thiele (19687).

This fungus was first recorded from New Zealand as *Cladosporium carpophilum* on peach (*Prunus persica* (L.) Batsch.), on apricot (*Prunus armeniaca* L.) by Cunningham (1921), and on cherry (*P. avium* L.) by Brien and Dingley (1955). Hughes (1953) showed that this species was characteristic of the genus *Fusicladium* rather than *Cladosporium.* Schweizer (1958) has shown that the *Fusicladium* sp. parasitising cherry is identical with *F. cerasi* (Rabenh.) Sacc., a distinct species with a perfect stage of *Venturia cerasi* Aderh. and differing morphologically from *Fusicladium carpophilum.*

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PASTURE ESTABLISHMENT ON A PODZOLISED SOIL IN NORTHLAND

I. Field trial studying lime and fertiliser requirements

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Summary

Investigations into fertiliser requirements for pasture establishment on Wharekohe silt loam have been made. No specific problems appeared, but the normal local fertiliser rates are too low. Lime at a minimum rate equivalent to 25 cwt of $CaCO_3$, 6 cwt of superphosphate, and 1 cwt of muriate of potash are suggested as an initial dressing. Subsequently additional phosphate is needed. With adequate fertiliser high pasture production and stocking rates are possible. Regrowth of manuka and rushes is thereby avoided.

INTRODUCTION

Clay and loam "gumland" soils, formed mainly under kauri (*Agathis australis* Salisb.) forest, occupy some 1,000 square miles in Northland, and are of several types (Taylor and Sutherland 1953). Wharekohe silt loam is a highly podzolised example, with thin grey topsoil overlying a light grey siliceous horizon which is often cemented (N.Z. Soil Bureau 1954). A considerable area of the more readily cultivated parts of this soil type has been developed to carry ewes or dairy stock. Ballinger (1953) has outlined recommended practices for developing this type of gumland, including cultivation, erosion control using contour furrowing, and fertiliser and lime requirements. Initial dressings of 3 to 4 cwt superphosphate or other form of phosphatic fertiliser are recommended, followed by two further applications in the first 12 months. The initial lime requirement is stated to be 1 to 2 tons per acre, and it is recommended that this should be followed by ½ to 1 ton in the following year, with subsequent annual applications of 5 cwt. It is also stated that potash is necessary on the highly podzolised soils.

Some pastures have established reasonably well on these soils, but many development blocks remain open, with considerable regeneration of manuka (*Leptospermum scoparium* Forst.) and rushes (*Juncus* spp.). As the new Divisional station includes a large area of

undeveloped gumland, it was considered opportune to study further pasture development on such soil, paying attention to the several nutritional and cultivation factors, as well as to seed mixtures of the latest pedigree varieties now available.

Experimental

Cultivation

The investigation was carried out at the Grasslands Division Station, D.S.I.R., Kaikohe. The area, with an average slope of 6° was covered by typical scrub (Fig. 1), mainly manuka and rushes. The soil type was predominantly Wharekohe silt loam, with a small area of the less strongly leached Waikare clay at the highest point. The area had been dug over for kauri gum, and there were numerous holes and remains of kauri trees.

The first cultivations were done with a light crawler tractor and bush and bog disc harrows. This equipment is lighter than that generally used. Cultivation began in mid-December 1956, and by mid-January 1957, when the first double cut with the discs had been completed, the scrub had been sufficiently crushed and dried to be burned. A second double cut with the discs followed the burn (Fig. 2). The land was then left until the following summer, as is normal practice, when two more double cuts were made (December 1957). Some levelling to fill gum holes, removal of the smaller kauri remains, and tandem discing followed by harrowing brought the land to a state ready for sowing. To counter washing of the soil between the time a relatively fine seed bed was prepared and pasture was established, graded banks were formed at intervals across the slope, with a fall of about 9 in. in 100 ft.

Fertiliser, Lime, and Inoculation Treatments

Treatments and times of application are given in Table 1. Subscripts give the rates, in hundredweights per acre, of the materials used. Individual plots were 2/5 acre each, and treatments were replicated twice in randomised blocks.

TABLE 1. Fertiliser, Lime, and Inoculation Treatments and Times of Application

Treatment No.	Fertiliser and Lime Applications, cwt per acre			
	Times of Application			Totals
	Autumn 1958	Spring 1958	Autumn 1959	
1	$L_{20}P_9K_0$ + inoculation	P_3	P_3	$L_{20}P_{18}K_0$
1X	$L_{20}P_3K_0$ — inoculation	P_3	P_3	$L_{20}P_9K_0$
2	$L_{20}P_9K_2$ + inoculation	P_6K_2	P_3	$L_{20}P_{18}K_4$
3	$L_{60}P_9K_0$ + inoculation	P_3	P_3	$L_{60}P_{18}K_0$
4	$L_{60}P_9K_2$ + inoculation	P_6K_2	P_3	$L_{60}P_{18}K_4$
4X	$L_{60}P_9K_2$ — inoculation	P_6K_2	P_3	$L_{60}P_{18}K_4$
5	$L_{60}P_3K_2$ + inoculation	P_3K_2	P_3	$L_{60}P_9K_4$

L = lime (68% $CaCO_3$); P = superphosphate; K = muriate of potash.

Fig. 1.—Part of the Establishment trial area before cultivations, showing the stunted manuka flowering. November 1954.

Fig. 2.—The same area as shown in Fig. 1, following initial cultivations and burning. March 1957.

Fig. 3.—The second application of fertiliser being applied on 12 November 1958, eight months after sowing. The area was photographed from approximately the same position as Figs. 1 and 2.

Lime (local, 68% $CaCO_3$), at 1 ton per acre, was sown on the trial area on 11 February 1958, and an additional 40 cwt of lime, where applicable, on 7 and 8 March 1958. Fertilisers were applied on 9 and 10 March 1958. The area was then chain harrowed and rolled with a Cambridge roller. Seed was sown on 20 March 1958, uninoculated seed first, covered with light chain harrows, and the area rolled again.

Seed was inoculated with double the recommended amount of a commercial clover inoculant* by dry mixing with the full seed mixture. A second application of fertiliser was made in spring 1958 between 13 and 27 November, and a third in autumn 1959 between 19 March and 30 April, as paddocks became available in the grazing rotation.

The $L_{20}P_3K_0$ treatment followed a common local practice, while Treatment 4 was considered to supply ample amounts of nutrients for establishment.

N. A. Clarke (pers. comm.), when Agricultural Instructor at Kaikohe, had observed a response to copper applications on similar adjacent land, and Wright and Gridley (1949) recorded a response to boron in pot trials. Copper sulphate and borax, each at 10 lb per acre, separately and mixed, were applied in strips 33 ft wide across the fertiliser plots.

* The inoculant was supplied by Biological Laboratories, Ltd., Auckland.

Seed Mixture

The seed mixture, given in lb per acre, was as follows:—

perennial ryegrass (*Lolium perenne* L.)	5
short-rotation ryegrass (*L. perenne* L. × *L. multiflorum* Lam.)	5
cocksfoot (*Dactylis glomerata* L.)	5
timothy (*Phleum pratense* L.)	2
subterranean clover (Mt. Barker) (*Trifolium subterraneum* L.)	4
red clover (cowgrass) (*T. pratense* L.)	4
white clover (*T. repens* L.)	2
	27

All species were of N.Z. pedigree origin, except subterranean clover. This mixture differed mainly from typical local mixtures in that it had a low ryegrass content.

Management

During the first winter plots were divided into two paddocks, each of 1/5 acre in Treatments 2, 3, and 5. Differences having failed to develop between Treatments 1 and 1X, and between 4 and 4X, the inoculation sub-treatments were ignored. This resulted in 2/5 acre paddocks for these two treatments. Rotational grazing with wethers of the four paddocks of each treatment began on 8 August 1958. To reduce transfer of dung and urine each of

Fig. 4.—The sward on $L_{20}P_3K_0$ treatment. This photograph was taken on 16 May 1958, two months after sowing, and shows the bare ground between unthrifty grass and clover plants.

the five treatments had its own flock. Sheep numbers were varied as necessary so that the grazing period of each paddock was the same for all treatments. Grazing continued until 29 June 1959, when measurements ceased.

Measurements

Pastures were scored at intervals for growth until grazing began. Pasture yields were then calculated from herbage clipped from frames of 7 ft × 3 ft. Two of these frames were put into each 2/5 acre paddock and one into each 1/5 acre paddock immediately before each grazing, and the herbage therein clipped when the sheep were moved. Sub-samples for dry matter determinations and botanical analyses were taken. No facilities were available for measuring live weight increases of the sheep, but average stocking rates have been calculated. Figs. 1, 2, and 3 show part of the area at three stages of development.

Results

Plant Responses to Fertiliser and Lime

Germination was good, and an even, early establishment of all sown species was obtained. Prior to the first scoring the only visible difference between treatments was markedly superior growth of both grasses and clovers in those paddocks which had received the higher rate of superphosphate. At no time was there

Fig. 5.—The sward on $L_{60}P_9K_2$ treatment, photographed on same day as Fig. 4. The vigour of both grasses and clovers, and the good ground cover, are clearly seen.

any clear response to boron or copper, and these areas have been ignored when making all measurements.

(a) *Growth Scores over Period 20.3.58 to 7.8.58*

These results are given in Table 2.

TABLE 2. Total Growth Scores of Grass and Clover, 0–5 Scale, Sown 20.3.58

Treatments	Dates and Total Scores					
	16.5.58		14.7.58		7.8.58	
	Grass	Clover	Grass	Clover	Grass	Clover
1. $L_{20}P_3K_0$	6	3	6	3	6	3
1X. $L_{20}P_3K_0$	6	3	6	3	6	3
2. $L_{20}P_9K_2$	9	7	8	8	8	9
3. $L_{60}P_3K_0$	10	8	9	5	8	5
4. $L_{60}P_9K_2$	10	10	9	9	9	8
4X. $L_{60}P_9K_2$	10	10	9	9	10	9
5. $L_{60}P_3K_2$	10	6	7	5	8	5
S.E. ±	0.4	0.8	0.6	1.0	0.4	1.0
Sig. Diff. (5%)	1.2	2.6	2.0	3.4	1.4	3.4

X = no inoculation.

In all cases Treatments 1 and IX (Fig. 4) were significantly poorer in both grass and clover than Treatments 2, 3, and 4 (Fig. 5). The only other significant difference was that Treatment 5 was poorer in clover than Treatment 4 at all dates. The initial observed increase in grass growth from the heavy superphosphate application had disappeared by the first scoring except that Treatment 1 remained poorer. Clover growth in all P_3 plots remained poorer than in P_9 plots throughout the scoring period. Potash application did not result in a significant increase in grass growth, but clover growth was significantly better at all times where potash had been applied together with the higher rate of superphosphate. Where adequate phosphate and potash had been applied the higher rate of lime did not result in any significant increase in either grass or clover growth.

(b) *Pasture Production over Period 20.3.58 to 22.6.59*

These results are presented in Table 3. Since no differences developed between inoculation sub-treatments these have been ignored.

From the start of grazing on 7 August the four paddocks of each treatment were grazed in rotation, and no true replication existed after this. Hence no statistical estimates of error were possible, and interpretation has been limited. In the first period

TABLE 3. HERBAGE YIELDS
(lb dry matter per acre)

Treatments	Periods			Total
	First: 20.3.58 * to 31.12.58	Second: 1.1.59 to 31.3.59	Third: 1.4.59 to 22.6.59	
1. $L_{20}P_3K_0$	2,600	1,300	1,000	4,900
2. $L_{20}P_9K_2$	7,300	3,400	2,100	12,800
3. $L_{60}P_9K_0$	4,500	2,200	2,100	8,800
4. $L_{60}P_9K_2$	7,300	3,900	2,200	13,400
5. $L_{60}P_3K_2$	4,900	3,300	2,400	10,600

Initial rates of application of fertiliser and lime are shown.
* Date of sowing.

all growth from sowing to the end of the first grazing was measured. At this time, in the presence of potash and the higher rate of lime, the higher rate of superphosphate (Treatment 4) gave a greatly increased yield over the lower rate (Treatment 5). Also in this period, in the presence of the higher rates of phosphate and lime,

TABLE 4. SPECIES YIELDS
(lb dry matter per acre)

Treatments	Species					
	Ryegrass	Cocksfoot	Timothy	Cow-grass	White Clover	Sub. Clover
	First Grazing Period: 8.8.58–31.12.58					
1. $L_{20}P_3K_0$	2,180	100	40	130	130	20
2. $L_{20}P_9K_2$	3,800	220	100	1,860	1,150	170
3. $L_{60}P_9K_0$	3,610	200	100	290	280	20
4. $L_{60}P_9K_2$	3,720	200	160	1,750	1,250	220
5. $L_{60}P_3K_2$	3,230	120	90	870	490	100
	Second Grazing Period: 1.1.59–31.3.59					
1. $L_{20}P_3K_0$	360	140	70	430	300	—
2. $L_{20}P_9K_2$	1,330	230	160	660	1,020	—
3. $L_{60}P_9K_0$	730	260	200	330	680	—
4. $L_{60}P_9K_2$	1,020	310	250	1,260	1,060	—
5. $L_{60}P_3K_2$	480	150	120	1,810	740	—
	Third Grazing Period: 1.4.59–22.6.59					
1. $L_{20}P_3K_0$	360	100	100	150	290	—
2. $L_{20}P_9K_2$	1,070	120	240	80	590	—
3. $L_{60}P_9K_0$	990	200	190	160	560	—
4. $L_{60}P_9K_2$	1,030	150	210	280	530	—
5. $L_{60}P_3K_2$	1,060	170	190	490	490	—

Initial rates of application of fertiliser and lime are shown.

the addition of potash (Treatment 4) resulted in a similar marked increase in yield compared with no potash (Treatment 3). In the presence of the higher rates of potash and phosphate, varying the rate of lime had no effect (Treatments 2 and 4). In the second period (Table 3) Treatment 5 improved considerably in comparison with Treatment 4, while Treatments 1 and 3, with no potash, remained relatively poor. In the third period (Table 3) pasture production from Treatment 1 was still well below that from any other treatment. Differences between the remaining treatments were small in this period.

Table 4 shows that in the first grazing period the higher rate of superphosphate (Treatments 2 and 4) gave a higher yield of all clovers than did the lower rate (Treatment 5), in the presence of adequate potash. Similarly the addition of potash (Treatment 4) at the higher superphosphate level resulted in a much higher yield of all clovers compared with no potash (Treatment 3). Increasing the rate of lime, at the higher rates of phosphate and potash, did not affect the yield of clovers. There was a marked improvement in clover yield at the lower phosphate level by the addition of potash and extra lime. Treatment 1 yielded less ryegrass than did the other treatments. These results conform to the growth scores recorded in Table 2.

In the second grazing period (Table 4), comparing Treatments 2 and 4, the higher lime application increased cowgrass production, and, comparing Treatments 3 and 4, the addition of potash similarly increased cowgrass. The highest cowgrass yield was in Treatment 5. White clover and ryegrass showed the same response to potash as did cowgrass (Treatments 3 and 4), but there was apparently no response to additional lime (Treatments 2 and 4).

In the third period (Table 4) no clear pattern of clover development appeared. Yield of ryegrass in Treatment 1 was markedly inferior to those of the other treatments.

Stocking Rates

Table 5 sets out the average stocking rates in the same periods as for herbage yields.

TABLE 5. Average Stocking Rates
(Wethers per acre)

Treatments	Period			
	8.8.58 to 31.12.58	1.1.59 to 31.3.59	1.4.59 to 22.6.59	8.8.58 to 22.6.59
1. $L_{20}P_3K_0$	3.4	3.6	4.4	3.7
2. $L_{20}P_9K_2$	9.8	10.4	10.3	10.1
3. $L_{00}P_9K_0$	6.4	6.6	9.1	7.2
4. $L_{60}P_9K_2$	10.7	14.5	12.5	12.3
5. $L_{00}P_3K_2$	6.8	10.6	8.4	8.3

Initial rates of application of fertiliser and lime are shown.

Stocking rates in Table 5 follow closely the herbage yields recorded in Table 3. The higher rate of superphosphate in Treatment 4 resulted in about 50% more stock being carried throughout the measurement period than was carried in Treatment 5. The beneficial effect of potash was shown in the comparison of Treatments 3 and 4, where the addition of potash resulted in nearly 70% increase in stock carrying. Treatment 4 with 60 cwt lime carried about 20% more stock than Treatment 2 with 20 cwt lime. Throughout the trial Treatment 1 carried least stock.

Discussion

Ballinger (1953) states: "Providing the land has been adequately dressed with lime and phosphate, pasture establishment is good and in a favourable season a paddock sown down in March will be ready to graze in two to three months". The results of the present trial confirm this statement, but help to specify more accurately what is an adequate dressing of fertiliser. The results indicate that 3 to 4 cwt of superphosphate at sowing are inadequate. They also confirm Ballinger's statement that on strongly podzolised "gumland" soils, such as Wharekohe, potash is very necessary.

The high initial demand for phosphate was confirmed in an adjacent area of 12 acres sown in March 1958, using the same seed mixture, and following the same cultivations. The following dressings per acre were applied: 55 cwt of lime, 7 cwt of superphosphate, 100 lb of muriate of potash, and 8 lb each of copper sulphate and borax. Grazing with sheep began on 7 July 1958. Pasture growth was comparable to that on the $L_{60}P_9K_2$ areas of the main trial. In November 1958 observational fertiliser plots were sited on parts of this area representative of Wharekohe and the less podzolised Waikare soils. In December these plots showed a marked response to superphosphate on both soils, while additional potash gave a small response only on the Wharekohe site. In November 1958 a further 3 cwt of superphosphate and 2 cwt of muriate of potash were applied to the whole area, and other observational plots were put down in March 1959. These plots showed no further responses to additional phosphate or potash.

There do not appear to be any problems of plant nutrition peculiar to Wharekohe silt loam, when the high demand for phosphate and moderate demands for potash and lime have been met. Jackman (1961) has found that boron and copper are the most likely minor elements to be rendered deficient by liming. In practice this is unlikely to happen during the establishment phase. In the present trial there was no evident effect on growth from applied copper and boron. Greenwood (1961) has shown that there are no soil properties hindering inoculation of red and white clovers, given adequate lime and fertiliser and a supply of suitable *Rhizobia*.

The results suggest that 2 tons of the lime used, 6 cwt of superphosphate, and 1 cwt of muriate of potash should be sufficient

for establishment, but that six months from sowing a phosphate deficiency will again exist. A further 3 cwt superphosphate will overcome this deficiency. Maintenance rates of application have not been determined. The trial does not distinguish between the response to phosphorus and sulphur in superphosphate, but Ballinger (1953) states that a non-sulphur phosphate fertiliser, basic slag, gives good results.

In the present trial differences between phosphate treatments disappeared as additional dressings were made to all plots. Although potash was not applied to all plots, differences between the no-potash and potash-treated plots gradually decreased. The reason for this is not known, but the topography of the area was such that all no-potash treatments had potash treatments above them, with the possibility of potash being washed downhill. Consequently it is not clear what rates of muriate of potash are needed after the initial application.

The use of greater dressings of lime than is usual may be a factor in better pasture growth in dry weather. There was a marked reduction in stocking in February 1959 in the $L_{20}P_9K_2$ treatment compared with $L_{60}P_9K_2$. This reduction followed a fortnight without recorded rainfall, and continued to early April 1959. Jackman (1961) has recorded soil pH values as being higher in the L_{60} plots than in the L_{20} plots at both the 0–3 in. and 3–6 in. depths. Van der Elst (1957) has shown the beneficial effects on growth of pastures during dry weather of deeper root penetration, when the pH of the deeper layers of peat was raised.

Seed mixtures vary in the district, but a typical mixture, in lb per acre, is: perennial ryegrass—20; short-rotation ryegrass—4; cocksfoot —4; timothy—2; crested dogstail—2; white clover—3; Montgomery red clover—2; subterranean clover—2; these components give a total mixture of 39 lb per acre. Paspalum is sometimes added to the mixture. This differs from that used in the trial mainly in the amount of ryegrass. It has been observed that many new pastures on similar soil are poor in clovers, but even with reasonable clover establishment a nitrogen-deficient period is common. Brougham (1954) showed that the degree to which clovers will grow successfully in autumn sowings is a function of grass seeding-rate. Sears (1953) demonstrated the vigorous early clover development in a soil that was low in available nitrogen but adequately supplied with other minerals. Poor clover establishment could be due to competition from grass seedlings, particularly with inadequate fertiliser applications. A nitrogen-deficient period could be emphasised by a high grass seeding-rate. The low rate of sowing of grass, particularly ryegrass, appeared to prevent undue competition between grasses and clovers. The expectation of producing relatively few sturdy grass plants was realised, and no marked nitrogen-deficient period occurred. The relatively few ryegrass plants may have been beneficial in reducing both nitrogen deficiency and competition with the clovers, which were able to make excellent early growth. These results show that the findings of Brougham (*loc. cit.*) and Sears (*loc. cit.*) apply also to Wharekohe silt loam. The adequacy

of the soil nitrogen may have been partly due to the fact that, once the soil is saturated, water movement appears to be mainly over the surface, and leaching may be small.

In some treatments cowgrass dominance occurred in the summer, possibly because of early undergrazing. While desirable for building soil fertility, this dominance may have retarded both white clover and grass development. Later differences in cowgrass yield could have been due to differences in grazing intensity.

There was no difference between inoculated and uninoculated plots. As suggested by Greenwood (1961), inoculation appears to have been by *Rhizobia* in the soil rather than being derived from the inoculum used. The applied *Rhizobia* could have perished under the dry conditions of sowing, particularly as the inoculum was dry-mixed with the seed, and the effective *Rhizobia* were those carried in on implements or by other means. Subterranean clover apparently failed to nodulate, and few plants survived after September 1958.

Where pasture growth was good very few seedlings of manuka or rushes were seen, and these were readily eaten owing to the high stocking rates. In the relatively poor open pasture of the $L_{20}P_3K_0$ treatment many seedlings of these weeds were seen. Some rush seedlings persisted, but all manuka seedlings were eaten. Even at the lowest stocking rate of the trial, stock concentration in an individual paddock subjected to this treatment, during the rotation, was about 14 sheep per acre. These rush seedlings persisted until the end of the trial. Two reasons for the ingress of rushes and manuka appear to be: (1) a weak sward following inadequate initial fertilising; and (2) stock concentration so low that the seedlings are not eaten at their palatable stage.

The adjacent 12 acre block carried 11 ewes per acre to the end of May 1959, 10 ewes per acre to the end of June, and 7 ewes per acre through the rest of the year. It has carried 10 ewes per acre through the winter of 1960. No supplementary feeding has been practised in either winter. A high potential production seems possible. The provision of graded banks across the slope appears to have reduced pugging by sheep, and the excellent winter carrying capacity could have been due in part to the small amount of pugging, as well as to the drier conditions for plant growth.

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PASTURE ESTABLISHMENT ON A PODZOLISED SOIL IN NORTHLAND

II. Plot trials studying nutrient deficiencies

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Summary

Trials were conducted on a strongly podzolised soil, Wharekohe silt loam, to determine nutrient deficiencies hindering the establishment of clovers. White and subterranean clovers were used as indicator plants.

Phosphate deficiency is acute, and 4 cwt per acre of superphosphate at sowing is inadequate.

High rates of lime are necessary to ensure good nodulation and vigorous early growth.

Liming can induce a boron deficiency and at higher levels a copper deficiency. The quantities of lime adequate to establish pastures successfully are not likely to induce a copper deficiency. Boron deficiency in white clover is not likely to appear after initial application of lime but may do so after subsequent liming.

Boron deficiency symptoms exhibited by the leaves, roots, and nodulation of subterranean clover are described. This clover is more susceptible to boron deficiency than is white clover.

Lime-pelleted clover seeds should be most effective in supplying adequate lime for good nodulation, without inducing a boron deficiency.

White clover can be successfully established, provided adequate phosphate, potassium, and lime are supplied. Subterranean clover is more difficult to establish, and the necessary conditions for successful establishment have not been defined.

A magnesium deficiency may appear after some years.

Introduction

In the first paper of this series Lambert (1961) decribed a field trial in which he studied lime and fertiliser effects on pasture establishment on a highly podzolised soil, Wharekohe silt loam. His range of treatments was limited, and it was considered desirable to extend this range using small plots. The object of these plot trials was to determine those nutrient deficiencies which could hinder the successful establishment of clovers. Since Wright and

Gridley (1949) had shown that mustard responded to boron when grown on limed Wharekohe sandy silt, and Cunningham (1954) has reported a copper deficiency in stock grazing on Wharekohe silt loam, it was evident that a study of minor elements was necessary.

Two small-plot trials were laid down in the autumn of 1958 to evaluate the effects of major and minor elements on the growth of white and subterranean clovers. In the autumn of 1959 a further trial was started to amplify the findings of the 1958 trials.

Experimental

I. 1958 trials

Materials and Methods

A relatively flat portion of the main field trial area was used. This was rotary hoed following cultivation, as described by Lambert (1961). White and subterranean clover were sown separately in pure stands and given the treatments enumerated below. The treatment subscripts refer to cwt per acre of the fertilising material used.

Trial 1. Major Elements

Treatments	
Basal	P_4 (superphosphate).
Potash and Lime	All combinations of K_0, K_1, K_2 (muriate), and L_0, L_{20}, L_{40} (Lime, 98% $CaCO_3$).
Additional Plots	1. $P_8K_2L_{40}$.
	2. $P_4K_2L_{20}N_1$ (urea).
	3. $P_4K_2L_{40}N_1$ (urea).

Trial 2. Minor Elements

Treatments	
Basal	1. $P_4K_2L_{20}$.
	2. $P_4K_2L_{40}$.
Minor Elements	1. $MgSO_4$, Mo, B, Cu, Zn, Mn. (All).
	2. All less Zn, Mn.
	3. All less Mo.
	4. All less $MgSO_4$.
	5. All less B, Cu.
	6. Nil.
Additional Plots	1. $P_4K_2L_{20}N_1$.
	2. $P_4K_2L_{20}N_1$. All minor elements.
	3. $P_4K_2L_{40}N_1$.
	4. $P_4K_2L_{40}N_1$. All minor elements.

The minor element materials were sodium molybdate (2½ oz.), borax (10 lb), copper chloride (10 lb), manganese sulphate-chloride, 2:1 (30 lb), and zinc chloride (5 lb) at the rates per acre indicated. Magnesium sulphate at 100 lb per acre was included. The lime was a high grade (98% $CaCO_3$), very finely ground "garden lime".

The area was divided into eight blocks, providing four replications of each species. Each block of each species was divided in half, and each half was randomly allotted, as in a split-plot design, to Trial 1 or 2. These main plots were then subdivided into 12 or 16 subplots to which the fertiliser combinations given above were applied.

On 20 March 1958 seed, inoculated with a commercial peat inoculum*, was broadcast. The lime and major nutrient fertilisers were then applied and the plots raked to cover the seed. Finally the minor elements were watered on.

The relative growth of the clovers was estimated visually using a 0–10 scale. This was done by independent observers. Each block comprising a replicate of both trials was scored as an independent unit.

Results

(1958)

Major Elements

A period of warm dry weather followed sowing, and conditions for growth were reasonably good. The early growth of the

TABLE 1. Total Scores* for White and Subterranean Clover Growth at 6/5/58, Trial 1

	White Clover					Subterranean Clover				
K	0	1	2			0	1	2		
L 0	97	79	45	221		53	42	48	143	
20	100	120	84 ±11	304	±19	78	42	56 ±10	176	±17
40	98	80	73	251		62	45	36	143	
	295	279	202			193	129	140		

	White Clover		Subterranean Clover	
Phosphate				
$P_4K_2L_{40}$	73		36	
$P_8K_2L_{40}$	97		61	
Nitrogen	N_0	N_1	N_0	N_1
$P_4K_2L_{20}$	84	75	56	41
$P_4K_2L_{40}$	73	41	36	26
S.E.	± 11		± 10	

* Four observers. Maximum possible score 160.

* The inoculum was kindly supplied by Biological Laboratories, Ltd.

subterranean clover was poor compared with that of the white clover. Growth was scored on 6/5/58, 25/6/58, and 25/8/58. Between the second and third scorings, when winter rains had fallen, the white clover was mown but there was insufficient subterranean clover growth to require cutting.

The results of the first scoring are given in Table 1.

Potash produced a highly significant decrease in the growth of both clovers. This effect was still evident at the next scoring, but by the third scoring had disappeared in the case of white clover, though still evident in the subterranean clover plots.

Lime (L_{20}) gave a highly significant increase in growth of white clover but not of subterranean clover. At both later scorings both levels of lime gave highly significant increases in growth of both clovers but there was no difference in effect between L_{20} and L_{40}.

From the comparison P_8 with P_4 (at K_2L_{40}) for both clovers, there was a significant increase due to the additional phosphate which was maintained at the third scoring. There was a significant reduction of the bulked clover scores due to urea, but this had disappeared by the second scoring.

Minor Elements

The results of the first scoring are given in Table 2.

TABLE 2. Total Scores for White and Subterranean Clover Growth at 6/5/58, Trial 2

Minor Element	White Clover			Subterranean Clover		
	L_{20}	L_{40}	$L_{20} + L_{40}$	L_{20}	L_{40}	$L_{20} + L_{40}$
All	98	90	188	106	110	216
— Zn — Mu	106	113	219	96	124	220
— Mo	118	103	221	109	122	231
— $MgSO_4$	119	82	201	110	114	224
— B — Cu	89	75	164	59	67	126
Nil	107	60	167	61	50	111
Nil + N_1	58	55	113	30	42	72
All + N_1	83	88	171	117	103	220
S.E.	± 11	± 11	± 15	± 10	± 10	± 14
Totals and S.E.	778	666	± 35	688	732	± 31

* Four scorers, maximum possible score 160.

Lime at the lower level (L_{20}) gave a significantly greater score for white clover than did L_{40} but with subterranean clover, level of liming did not have any effect. Nitrogen significantly depressed growth in the absence of minor elements but had no effect in their presence, and this situation remained at the later scoring dates.

Where no minor elements were applied and at both lime levels, the score for subterranean clover was significantly less than where all minor elements had been applied. In the case of white clover this effect was significant only at the high lime level. The subterranean clover results show that this reduction was due only to boron and/or copper. The same effects due to boron and copper were found at both later scorings but were greatly reduced in the case of the third scoring of white clover. At all scorings the depression due to inadequate boron and/or copper was less severe in the case of white clover.

A pot trial was then conducted to determine if boron and/or copper were responsible for the enhanced growth in the limed soil.

Treatments

Basal

KH_2PO_4 and $CaSO_4$ to give P and SO_4 equivalent to 8 cwt of superphosphate per acre.

$CaCO_3$ to give 75% and 100% base saturation. The C.E.C. of the soil was 6.0 and the T.E.B. 0.85 m.e. per 100 g soil.

Minor Elements

(1) Nil.
(2) Borax at 10 lb/acre.
(3) $CuSO_4.5H_2O$ at 10 lb/acre.
(4) (2) + (3).
(5) Minor element mixture as used in the plot trial.

Half-gallon brown glass bottles were cut in two and the top inverted into the cut-off bottom portion which acted as a drip collector. The tops held 1.2 kg of air dry soil. The lime was mixed throughout the soil but the phosphate was kept in the top inch. The minor elements were watered on, and glass distilled water (Ballentine 1954) was added to saturation. There were six replicates. After 14 days 20 inoculated, small, subterranean clover seeds of uniform size were planted (18/8/58) in each pot. Later

TABLE 3. Dry Matter Yields of Subterranean Clover Growing With and Without Minor Elements

Minor Element	Lime Level	
	75% Saturation	100% Saturation
	g/pot	g/pot
Nil	2.74	2.00
Cu	2.27	2.91
B	3.29	3.49
Cu + B	3.35	3.95
All	3.47	3.79
S.E.	± 0.19	

the plants were thinned to five per pot. The herbage was harvested after 35 and 59 days from planting. After the second harvest the plants were dug up and the roots and nodules examined.

The dry matter yields are given in Table 3. Copper significantly reduced growth at the lower lime level but significantly increased growth at the higher level of liming. Boron significantly increased growth at both levels of lime. Copper plus boron did not differ from boron alone at the lower level of lime but produced a significant increase in growth over that from boron alone at the higher liming level. The minor element mixture (Trt. 5) had no effect beyond that due to boron and copper.

Boron deficiency was accompanied by striking vegetative and root abnormalities.

The *leaf* symptoms, which are described below, appeared in the order given:—

(a) Unifoliate leaf. Small, pointed, cupped, and of a dark blue-green colour except at the edges which appeared lighter partially owing to a concentration of hairs. Petiole short. (Fig. 1).

(b) Early trifoliate leaves. Small, pointed, cupped, dark blue-green in colour. Petiole short.

(c) Cotyledons. Initially green but changing to bronze and then blood red.

Fig. 1.—Boron deficiency in subterranean clover seedlings. Right-hand seedling grown with added boron, other seedlings without.

(d) In some cases normally shaped leaves become blood red in colour.

The *roots* of boron deficient plants were brown with short stubby laterals. The brown colour was accentuated by copper when applied to the low- but not the higher-limed pots.

The effectiveness of the inoculum used was dependent upon boron being present. With lime alone nodulation was poor, and copper with low lime accentuated this situation. With boron present nodulation was satisfactory, and was characterised by groups of nodules just below the crown, indicating that infection had been by organisms from the inoculum. In some cases plants without boron had made a poor start but later made good growth and developed large and healthy root systems. In these cases there was no infection near the crown. (Fig. 2.)

Fig. 2.—Boron deficiency and nodulation of subterranean clover. Right-hand plant grown with added boron, left-hand plant without.

Soil Measurements

The exchange status of a profile from an unfertilised and unlimed but cultivated area close to the small plot trial is given in Table 4. Ten cores, 2 ft deep, were bulked. The top of the A_2 horizon was obvious in all cases but the division between A_2 and B was not clearly evident in the cores and appeared to vary from core to core. For this reason the 12–20 in. samples were probably mixtures of A_2 and B horizons. The results show the very low exchangeable cation status of this soil. The magnesium supply is relatively better than that of the other nutrient elements.

TABLE 4. C.E.C. and Exchangeable Cations in a Profile of Wharekohe Silt Loam*

Soil Depth (in.)	0–4	4–8	8–12	12–16	16–20	20–24
Horizon	A_1	A_1	A_2	A_2–B	A_2–B	B
C.E.C. m.e. %	8.3	6.6	4.3	9.2	13.8	18.1
Ca "	0.47	0.42	0.20	0.31	0.58	0.83
Mg "	0.49	0.19	0.11	0.26	0.37	0.63
K "	0.08	0.06	0.06	0.05	0.05	0.06
Na "	0.17	0.06	0.05	0.09	0.14	0.19
% Saturation	14.6	11.1	9.8	7.6	8.3	9.5

* Leached with *N* ammonium acetate, pH 7.

A sample of topsoil was sent to the State College of Tropical Agriculture, Deventer, The Netherlands. Of particular interest was their report that the P_2O_5 soluble in 2N H_2SO_4 was only 0.008%.

In Table 5 are given cation exchange measurements of samples taken from Lambert's (1961) field trial (sampled 2/10/58) and the small plot trial (sampled 12/12/58). Despite free lime where 2 tons had been applied to the plot trial the results show that liming effects must have been largely confined to the top 3 in. of soil even 9 months after application.

TABLE 5. Cation Exchange Measurements* of Limed and Unlimed Wharekohe Silt Loam

Treatment	Soil Depth	C.E.C.	T.E.B.	pH
	in.	m.e. %	m.e. %	1 : 2.5 soil/water
Field Trial (Lambert)				
Lime 68% $CaCO_3$				
1 ton	0–3	7.2	2.7	5.2
	3–6	5.7	0.7	4.6
3 tons	0–3	9.5	6.3	5.9
	3–6	7.5	1.7	5.0
Plot Trial (1958)				
Lime 98% $CaCO_3$				
Nil	0–3	8.6	2.0	4.9
	3–6	7.6	1.4	4.6
1 ton	0–3	7.6	5.2	5.7
	3–6	8.0	1.5	4.6
2 tons	0–3	8.5	11.3**	6.5
	3–6	7.4	2.0	4.7

* Leached with N. ammonium acetate, pH 7.
** Free lime present.

Discussion

(1958 Results)

Phosphate

Those plots which had received P_8 made better growth than those which received P_4, and 18 months after sowing this difference was very evident. Growth on the P_8 plots was very good but was generally poor on the P_4 plots. These results confirm those of Lambert (*loc. cit.*) and indicate that 3–4 cwt of superphosphate was inadequate for vigorous clover establishment.

Potash

The failure of potash to produce additional growth, as was obtained by Lambert (*loc. cit.*), was probably due to inadequate phosphate and a deficiency of boron induced by the heavier liming of the small plots. Potash caused a depression of growth. This unexpected result may be explained in terms of the "salt effect" of Mattson *et al.* (1949) deduced from Donnan distribution considerations. Mattson *et al.* (*loc. cit.*) postulate that the distribution of a number of ions, including $H_2PO_4^1$, between cation exchangers of differing exchange capacity is such that the exchanger having the lowest capacity will possess the greatest activity of the ion in its micellar solution and that this effect will be greater as the relative overall ion activity increases. In the present case the soil had a low cation exchange capacity (7–9 m e./100 g), while clovers have a high root exchange capacity (Drake *et al.* 1951). The "salt effect" could have operated, since for about six weeks after planting there was little rain. Between the second and third scorings the white, but not the subterranean, clover was mown. The disappearance of the depression of white clover could have been due to the winter rains having removed soluble salts, while the apparent continuation of the depression of the subterranean clover was probably due to differences established early and not eliminated by mowing, as in the white clover. It is probable that the potash depression was due to a salt effect aggravating a phosphate deficiency.

Lime

As would be expected lime was found to be essential for vigorous establishment of clover, but there was no clear evidence that 2 tons were superior to 1 ton at this stage of growth.

Minor Elements

The results of the pot trial show that liming can induce deficiencies of both boron and copper in subterranean clover. The effects upon both clovers observed in the field were probably due to a boron deficiency which appears to operate in two ways. Firstly, boron deficiency inhibits nodulation; and, secondly, the deficiency had a direct effect on the plants. The regrowth of white clover, after mowing, was not greatly affected by boron deficiency. This was probably due to the fact that by this time the roots had penetrated below the limed zone of soil (Table 5) and were able to

obtain sufficient boron in this lower zone. This explanation was suggested by the observation that some plants had stunted lateral branches at the top of the main root but more distally the root system had proliferated strongly. Nodulation was on this distal zone but not close to the crown. It appears likely that the boron deficiency exerted its main influence very early in the life of the clovers. Lambert (1961) did not observe any response to boron in his field trial receiving 3 tons of local lime. It is quite possible that there was no response owing to the fact that he used relatively coarsely ground limestone which would tend to give a mosaic of high and low lime concentrations. In the present plot trials, a finely ground, almost powdery limestone was used, which would tend to produce a more uniform depression of available boron in the topsoil.

No other minor element produced any effect. It is not understood why urea depressed growth in the absence of minor elements, but had no effect in their presence. A possible explanation is that the additional nitrogen induced a manganese deficiency within the plants as has been noted by Heintze (1946) in the case of peas treated with nitrate nitrogen.

II. 1959 TRIAL

Materials and Methods

The objects of this trial were:

(1) To determine whether the recovery from the minor element deficiency was due to root penetration below the limed layer of soil.

(2) To obtain evidence as to whether poor initial growth was related to nodulation, minor element deficiency, and level of liming.

Treatments

Basal	P_6K_1 (superphosphate, muriate).
Lime	(1) L_{20} (98%). 98% $CaCO_3$ lime applied to the surface.
	(2) L_{30} (98%). The same lime worked into the top 3 in. by rotary hoe.
	(3) L_{20} (68%). 68% $CaCO_3$ local lime similarly worked into the top 3 in.
	(4) L_{40} (68%). The same lime similarly applied.
	(5) L_{60} (68%). The same lime similarly applied.
Minor Elements	(1) Nil.
	(2) Borax at 10 lb per acre.
	(3) Borax and $CuSO_4 .5H_2O$ each at 10 lb per acre.

It had been planned to use the same "garden lime" as previously employed, but more coarsely ground agricultural lime from the same source and of the same $CaCO_3$ content was eventually used.

White and subterranean clover, each with ryegrass, were sown. It was considered that, since nodulation was an important factor, differences due to treatment would be more evident if the soil nitrogen were reduced by the grass.

Each treatment was replicated four times. A 5 × 3 split-plot design was used for each clover species, the two trials being interwoven by sowing each plot with both clover species. Both clovers were inoculated with a proven inoculum. Seed and fertiliser were sown on 17/4/59, using the same methods as for the previous trials.

Results

(1959)

The first scoring of relative growth was made eight weeks after sowing. At the same time approximately 10 plants per sub-plot (120 plants per lime treatment) were dug up and the nolulation assessed.

TABLE 6. Total Scores* for White and Subterranean Clover Growth in the 1959 Trial at 17/6/59, together with Soil pH

	White Clover				Subterranean Clover				Soil pH	
Minor Elements	O	B	B+Cu	Total	O	B	B+Cu	Total	Depth	pH
Lime										
L_{20} (68%), 14 cwt $CaCO_3$	27	35	28	90	27	36	24	87	0–3	5.3
L_{20} (98%), 20 cwt $CaCO_3$	35	41	33	109	43	44	31	118	0–1½ 1½–3	5.8 4.6
L_{40} (68%), 27 cwt $CaCO_3$	41	50	38	129	33	39	38	110	0–3	6.0
L_{80} (98%), 29 cwt $CaCO_3$	59	52	50	161	50	58	60	168	0–3	5.8
L^e_0 (68%), 41 cwt $CaCO_3$	50	50	44	144	48	56	49	153	0–3	6.5
S.E.		± 4		± 17		± 5		± 11		
Totals	212	228	193		201	233	202			
		± 10				± 10				

* Two scorers, maximum possible score 80.

Growth scores are given in Table 6. Copper significantly depressed the growth of both clovers. Boron significantly improved the growth of subterranean clover, but the effect on white clover failed to reach significance. In the analysis of total clover scores, the effects of boron and copper on both clovers were significant; but the effects were small compared with that due to boron plus copper in the 1958 trial. This was probably due to the difference in fineness of grinding of the limes which were used. The agricultural lime, relatively coarsely ground, used in this 1959 trial would be expected to produce a mosaic of high- and low-limed spots, and presumably the plants were obtaining nearly adequate amounts of boron from these low-lime spots. There was a highly significant increase in growth of both clovers with increasing level of lime.

TABLE 7. Percentages of Plants (1) Effectively Nodulated, (2) Effectively Nodulated and with Nodules Clumped Below the Crown, 1959 Trial

Lime Treatment	Nodulation Type	White Clover	Subterranean Clover
		%	%
L_{20} (98%)	1	100	97
	2	98	97
L_{30} (98%)	1	100	93
	2	99	88
L_{20} (68%)	1	79	59
	2	37	35
L_{40} (68%)	1	94	81
	2	82	45
L_{60} (68%)	1	98	95
	2	91	81

In Table 7 are given the results of the nodulation assessment. Since minor elements had no effect at all, the results are limited to the lime effects averaged over the minor element treatments. There were significant improvements in nodulation with increasing level of lime. The success of the inoculum, as judged by the frequency of appearance of a clump of nodules just below the crown, was also related to level of liming. Plants not nodulated, or ineffectively nodulated, were more numerous the lower the liming level, with a maximum of 41% of the subterranean clover plants on plots receiving only one ton of the local lime. The successful nodulation of subterranean clover was more dependant upon level of lime than was that of white clover. The effect of the surface application of one ton of the high grade lime was outstandingly good as compared with the other treatments. This was probably due to the fact that at the time of infection this surface treatment ensured the highest concentration of lime round the seed. From the pH measurements there is no indication that this lime had moved below 1½ in. from the surface (Table 6).

General Discussion and Conclusions

(1958 and 1959 Trials)

The results show that multiple deficiencies of both major and minor nutrient elements exist in this soil.

Wells and Saunders (1960) have shown that, with increasing weathering of the yellow brown earths (Taylor and Cox 1956), the phosphate content falls and the phosphate retention capacity increases but when the extreme podzolised state is reached (Wharekohe silt loam) both content and retention capacity are small. It is of interest that the 0.008% P_2O_5 found in the sample of topsoil sent to Deventer (Netherlands) was the lowest phosphate content they had ever recorded. In their experience the poorest tropical soils would be expected to contain 0.03% P_2O_5. These results agree with the present evidence that initially there is a very large phosphate deficiency in this podzolised soil. Three to four cwt per acre of superphosphate is inadequate. The small phosphate retention capacity found by Wells and Saunders (*loc. cit.*) indicates that, if the initial deficiency is rectified, maintenance dressings thereafter should be small.

The present trials provide no information on potash other than indicating that using a potassic fertiliser can aggravate a phosphate deficiency under dry conditions. The soil analyses show a low available supply.

The trials have not defined an optimum level of lime. High rates appear necessary to ensure nodulation and growth, but such can induce a boron deficiency. This problem can probably be solved best by using lime-pelleted clover seeds which are now available commercially. The local lime is relatively low in carbonate, and relatively high rates can probaby be used initially without danger of inducing a boron deficiency in white clover. However, a boron deficiency may appear when lime is applied subsequently, owing to a more uniform rise in soil pH and to greater depth of lime penetration. It is unlikely that a copper deficiency will occur unless lime is used in large amounts, but the evidence of a marginal supply supports the practice of spraying pastures with copper for reasons of stock health.

The soil analyses (Table 4) show that the magnesium supply is probably the least critical amongst the major nutrient elements. The reserves must, however, be small, and responses to magnesium are likely to appear in the future, especially in areas producing well as a result of heavy fertilisation with superphosphate and potash.

White clover was successfully established in these trials, but subterranean clover was, in general, a failure. Lambert's (1961) trial showed the same contrast, whereas Greenwood (1961) obtained good subterranean clover growth. Nodulation of subterranean clover appears to be more dependent on adequate lime and boron supplies than is that of white clover. The fact remains, however, that the requirements for the successful establishment of subterranean clover in this soil have not been adequately defined.

The potentialities of this soil appear to be good. Manawatu silt loam from Palmerston North, acknowledged to be a highly productive soil, was taken to the experimental area and used to make a 6 in. deep plot. This was planted to the two clovers. These grew well, but the white clover growth in the best plots of the trial, though developing more slowly, eventually equalled that on the Manawatu soil.

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PASTURE ESTABLISHMENT ON A PODZOLISED SOIL IN NORTHLAND

III. Studies on rhizobial populations and the effects of inoculation

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Summary

Factors affecting rhizobial populations and the nodulation of two clover and two lotus species in a strongly podzolised soil, Wharekohe silt loam, have been examined.

No clover nor lotus rhizobia were detected in this infertile acid soil in its undisturbed state. After thorough cultivation lotus rhizobia were found in all samples, and clover rhizobia in samples from areas which had been limed. The clover rhizobia were largely ineffective on subterranean clover.

In small plots of cultivated soil it was found that:

(a) Inoculation was advisable for white clover, and essential for subterranean clover.

(b) Lime was essential for adequate nodulation of white clover, subterranean clover, and *Lotus corniculatus*, and beneficial for *Lotus uliginosus*. Lime-pelletted clover seed gave good initial nodulation.

(c) At the level of liming used, a micronutrient deficiency, apparently of boron, was induced, and addition of a micronutrient mixture increased nodulation and greatly improved nodule form and plant growth.

In nearby inoculated field sowings, good nodulation occurred with red and white clover, but with subterranean clover most of the nodules were found to be ineffective.

Introduction

In the first two papers of this series (Lambert 1961, Jackman 1961) experiments were described in which the fertiliser requirements for successful pasture establishment on a strongly podzolised soil, Wharekohe silt loam, situated at the Kaikohe substation of Grasslands Division, D.S.I.R., were studied. One aspect not specifically studied in these experiments was the nodulation of the clovers. A prior test on a sample of the undisturbed gumland soil

had shown that clover and lotus rhizobia were apparently absent, and it was not known how readily these rhizobia would establish in this infertile acid soil after it had been cultivated and limed, and whether any difficulties were likely to be experienced in obtaining good early nodulation from inoculated seed. Consequently, in order to obtain information on these points, a small plot inoculation experiment was laid down using two clover and two lotus species, together with a small series of unsown plots with different combinations of fertilisers, and rhizobia added. In this paper results of these experiments are given, together with some determinations on nodulation in the field sowing (Lambert 1961). .

Experimental

The experiments reported here were carried out during the months of March to June 1958. The area used for the experimental plots was located in the far corner of the field trial area, adjacent to undeveloped gumland. Initial cultivation was the same as for the rest of the area (Lambert 1961), but no lime nor fertiliser was applied. To prevent any chance of wash from the higher ground that had been limed, a channel was cut above the plot area.

Experiment 1

The first experiment was a factorial small plot experiment with three replications. The plots were 6 ft by 2½ ft, with 1½ ft pathways between plots. All plots received a basic dressing of superphosphate at 4 cwt per acre and muriate of potash at 2 cwt per acre.

Three lime and micronutrient treatments were applied:—

(a) No lime, no micronutrients.

(b) Finely ground "garden lime" 98% $CaCO_3$ (Jackman 1961) at 1½ tons per acre, no micronutrients.

(c) Lime as in (b) plus the full micronutrient mixture, together with magnesium, as used by Jackman in his initial small plot fertiliser experiment (Jackman 1961). This mixture contained manganese, boron, copper, zinc, and molybdenum.

All plots except those of Treatment 8 had one 6 ft row each of subterranean clover (*Trifolium subterraneum* L.), white clover (*T. repens* L.), autotetraploid *Lotus uliginosus* Schkuhr. (Barclay 1957), and *Lotus corniculatus* L. "Viking".

Inoculation Treatments

1. No inoculation.
2. Seed inoculated with skim milk as sticker.
3. Seed inoculated as in (2), but with urea added to the soil prior to sowing.
4. Seed inoculated as in (2) but with a light dusting of lime added to the seed after inoculation.

5. Seed inoculated as in (2) but with molasses added as sticker, followed by a light dusting of lime.
6. Seed inoculated at about five times the rate of inoculum with skim milk, molasses, and a dusting of lime.
7. Uninoculated seed used, but with an inoculum of sifted Manawatu soil sprinkled along the rows at approximately $\frac{3}{4}$ lb per plot.
8. Lime-pelletted seed of subterranean and white clover used, pellets inoculated as in (2). Each plot had one row each of subterranean and of white clover in hard and in soft pellets.

All the above inoculation treatments were used in plots of no lime (a) and lime (b), but only Treatments 1, 2, and 3 in plots of lime and micronutrients (c).

Inoculation

The strain of clover Rhizobium used (NZP 1) was the same as that used in the inoculation experiments in pumice soils (Sears *et al.* 1955). It had been found effective on white and subterranean clovers, and also on a number of other clover species. An inoculum of this organism grown on a peat-pumice mixture was used, containing approximately 10^9 viable cells per g, the inoculum being added at approximately 1% of the weight of seed. For the Lotus species a mixture of two rhizobial strains was used: NZP 2002 isolated from *L. uliginosus* growing at Palmerston North and NZP 2014 isolated from *L. corniculatus* growing on soil from Kaikohe. Both had been found effective in tube tests with both Lotus species. The inoculum was prepared from agar slopes of these strains.

The Manawatu soil used as an inoculum in Treatment 7 had been collected from near white clover and lotus plants. It was found to contain approximately 70,000 clover rhizobia and 700,000 lotus rhizobia per g. Most of the clover rhizobia were ineffective on subterranean clover.

The subterranean clover seed used for the control plots was shaken with 70% ethyl alcohol and dried before being measured out because it was rather dusty and thus might have carried some rhizobia.

Sowing

The plots were laid out and fertilised on 19 March and sown the following day. At sowing, the soil was dry at the surface, but moist underneath. The seed bed could therefore be prepared satisfactorily by the use of a rake. The fertilisers were raked into the top inch or so of soil in the plots, urea and micronutrients being added in solution. Four seed rows were made in each plot with a rake, and the seed sown and covered in. Care was taken at sowing not to spread inoculum from one plot to another.

Germination was satisfactory, but bird damage to the seedlings caused some patchiness in the rows. Up till the time of the first harvest (8 May) rainfall was light and growth was slightly restricted by lack of moisture. Shortly before the second harvest (26 June) heavy rain fell, saturating the top soil. Although cross-contamination between plots was thus possible due to water movement down slope through and over the soil, it would have been too recent to have affected the results of the second harvest.

Harvests

On 8 May visual scorings for vigour of growth of subterranean clover and *L. uliginosus* were made by four observers, and half the plants in each row of subterranean clover and all plants in the white clover rows were dug up for nodule observations. The lotus plants in many plots were too small to harvest.

On 26–27 June visual scoring for vigour of growth of *L. uliginosus* was made by two observers, and the remaining subterranean clover plants and all the plants of *L. uliginosus* and *L. corniculatus* were harvested for nodule observations.

Experiment 2

Experiment 2 consisted of work on a non-replicated series of plots of the same size as in Experiment 1 and laid down at the same time, with different combinations of fertilisers at the same rates as in Experiment 1. No seed was sown in the plots, but all plots except plot 6 were inoculated with clover and lotus rhizobia from agar slopes. The rhizobia were applied as a mixed suspension in water, at a rate estimated to be between 10^7 and 10^8 viable cells of each organism per sq. ft.

The treatments were as follows:

Plot 1. No fertiliser, inoculated as above.

Plot 2. Superphosphate and potash, inoculated.

Plot 3. Lime, inoculated.

Plot 4. Superphosphate, potash, and lime, inoculated.

Plot 5. Superphosphate, potash, lime, and micronutrients, inoculated.

Plot 6. Superphosphate, potash, lime, and micronutrients, but not inoculated.

Plot 7. Superphosphate, potash, and lime, inoculated. This plot was, however, situated in the undeveloped scrub, more than one chain from the edge of the cultivated area, and was hand cleared and dug over at the time of laying down the experiment.

At various times soil samples, each consisting of 2–3 plugs ($\frac{5}{8}$ in. diam., 2 in. deep), were taken from the plots and elsewhere (as shown in Table 4) with a small freshly sterilised soil sampler, and populations

of clover and lotus rhizobia determined. The method of counting consisted of adding a series of 10-fold dilutions of the soil suspensions to sterile tubes containing young seedlings of subterranean clover and *L. uliginosus* and later examining the plants for nodules, the number of rhizobia in the soil sample then being calculated (Fisher and Yates 1953). Three replicate tubes at each dilution were normally used; this gave approximate limits (one standard error either side of the mean) of half and twice the calculated count. Since sampling errors could also be large, only differences in count of about one hundredfold or greater were taken as being real. With soil samples in which the rhizobia were expected to be few or absent the lowest dilution used was normally 2.5g soil per tube.

Nodulation of Seedlings from Field Sowings (Lambert 1961)

In the field sowings the lowest and highest fertiliser treatments were $L_{20}P_3K_0$ and $L_{60}P_9K_2$, these symbols designating rates of lime, superphosphate, and muriate of potash, respectively, in hundredweight per acre. Separate plots of these treatments were put down with uninoculated and with inoculated seed, only inoculated seed being used for the other fertiliser treatments of the experiment. All plots were crossed with boron and copper treatments. The lime used was impure (68% $CaCO_3$) (Jackman 1961) and lumpy, and was applied some time before sowing. For the inoculation a commercial inoculant* was used, applied dry to the seed at twice the recommended rate.

The above arrangement of plots made it possible to determine whether there were any effects on nodulation from the inoculation or from the boron plus copper treatment. For this purpose, on 29–30 May, 25 seedlings each of subterranean clover, white clover, and red clover were dug up on a planned layout from the no micronutrient section and from the boron plus copper section of each plot of the above two fertiliser treatments, both from the plots with and those without inoculation. These seedlings were examined for nodulation, the presence of nodules, their position and apparent effectiveness being recorded.

A sample from the same batch of inoculant, when tested by the dilution method using subterranean clover, was found to contain approximately 10^9 effective rhizobia per g, with no indication of any rhizobia ineffective on subterranean clover. Other organisms were present, preventing colony counts, but these did not appear to affect adversely the nodulation of subterranean clover in tubes.

Results

Experiment 1

The results of the visual scoring made on 8 May (first harvest) of the growth of subterranean clover in Experiment 1 are given in Table 1. Over this period the available nitrogen in the soil proved to be sufficient to meet the requirements of the plants, sown as they

* This inoculant was supplied by Biological Laboratories, Ltd, Auckland

were in well-spaced rows. Thus the plants were not dependent on fixation of nitrogen for growth, so that differences did not necessarily bear any relation to nodulation differences.

TABLE 1. Scoring of Subterranean Clover Growth in Experiment 1 at First Harvest

Treatments	(a) No Lime	(b) Lime	(c) Lime and Micronutrients
1. No inoculation	68 ± 7	36 ± 8	86 ± 7
2. Inoculated	75	36	88
3. Inoculated, urea added	78	26	95
4. Inoculated, with lime	75	34	
5. Inoculated, with molasses and lime	72	31	
6. Inoculated at high rate	69	32	
7. Inoculated with Manawatu soil	60	30	
8. Inoculated pelletted seed			
8h—hard pellets	88	93	
8s—soft pellets	93	34	
Sig. diff. at 5%	20		

(Maximum possible score—120)

The addition of lime caused a significant growth depression in all treatments except 8h. However, where micronutrients were added as well as lime in Treatments 1–3, there was a significant increase in growth over the corresponding no lime plots. Growth in these plots with lime and micronutrients was, in fact, excellent by any standard, and quite as good as that in an observation plot nearby in which the topsoil had been removed and replaced by 6 in. of Manawatu silt loam topsoil. This growth depression, considered by Jackman (1961) to be a boron deficiency induced by the liming, was present in all species, and most marked in the subterranean clover. By 26 June the growth depression was, by observation, less marked in the remaining subterranean clover plants, and was no longer clearly visible in *L. uliginosus,* but the response to lime plus micronutrients was still marked in both species.

The percentages of nodulated plants of subterranean clover and white clover harvested on 8 May (first harvest) are given in Table 2. There were big variations in percentages between replicate plots, and only the major differences were significant.

In the plots without lime nodulation was negligible for both species without inoculation, and inoculation alone gave only low percentages of nodulated plants. The lime dusted on the seed in Treatments 4a and 5a gave a significant increase over Treatment 2a with subterranean clover, but no increase with white clover, and for both species there was a further increase significant at the 10% level due to the higher rate of inoculation. Manawatu soil as an inoculum gave a high percentage of nodulated plants with white

TABLE 2. Percentage of Nodulated Plants of Subterranean and White Clovers at First Harvest of Experiment 1

Treatments	Subterranean Clover			White Clover		
	(a) No Lime	(b) Lime	(c) Lime + Micro-nutrients	(a) No Lime	(b) Lime	(c) Lime + Micro-nutrients
1. No inoculation	1±2	3±5	67±15	1±2	7±5	29±12
2. Inoculated	10±8	42±15	92±9	10±5	55±13	68±12
3. Inoculated, urea added	23±10	22±10	66±15	4±3	23±10	64±12
4. Inoculated, with lime	43±16	60±16		10±7	53±12	
5. Inoculated, with molasses + lime	52±16	34±16		7±6	43±13	
6. Inoculated at high rate	85±11	57±16		28±11	52±12	
7. Inoculated with Manawatu soil	10±9	45±16		30±11	88±9	
8. Inoculated pelletted seed						
8h—hard pellets	75±13	95±7		42±12	77±11	
8s—soft pellets	98±4	63±16		79±11	74±12	

clover but not with subterranean clover. Also many of the subterranean clover nodules in this treatment appeared ineffective. Lime-pelletted seed gave very good nodulation with both species.

In the plots with lime added, inoculation gave a significant increase in nodulation, with little or no added effect from the different additions or higher rate of inoculation, and again the pelletted seed gave very good nodulation. This effect of lime in increasing nodulation was in marked contrast with its growth-depressing effect.

The addition of micronutrients as well as lime in Treatments 1–3 resulted in a highly significant increase in the percentage of nodulated plants, and the nodules were also much larger and of better colour. Of interest also was the high nodulation of the uninoculated plots, especially in the case of subterranean clover. This was not primarily due to contamination from the other plots, as was shown by the ineffective appearance of most of the nodules on the subterranean clover in these uninoculated plots, in contrast with the predominantly effective nodules in the inoculated plots.

The percentages of nodulated plants of subterranean clover, *L. uliginosus* and *L. corniculatus* harvested on 26–27 June (second harvest) are given in Table 3.

No marked nitrogen deficiency was observed in the plants without nodules.

Subterranean clover will be discussed first. Further nodulation had occurred, and in all treatments which received any lime almost all plants were nodulated. However, the effect of lime on the form of the nodules and roots was very striking. In unlimed soil [Treatment (a)] the nodules were generally few, often only one per plant, large but of rather poor colour, and confined to just

TABLE 3. PERCENTAGES OF NODULATED PLANTS OF SUBTERRANEAN CLOVER, *Lotus uliginosus*, AND *Lotus corniculatus* AT SECOND HARVEST OF EXPERIMENT 1

Treatments	Subterranean Clover			*Lotus uliginosus*			*Lotus corniculatus*		
	(a) No Lime	(b) Lime	(c) Lime and micro-nutrients	(a) No Lime	(b) Lime	(c) Lime and micro-nutrients	(a) No Lime	(b) Lime	(c) Lime and micro-nutrients
1. No inoculation	0	94	100	53	96	100	2	48	87
2. Inoculated	8	100	100	46	99	99	5	62	86
3. Inoculated, urea added	84	100	100	63	98	98	7	36	84
4. Inoculated, with lime	71	98		71	86		12	58	
5. Inoculated, with molasses and lime	86	100		64	98		7	52	
6. Inoculated at high rate	98	100		77	97		21	64	
7. Inoculated with Manawatu soil	17	99		66	99		7	84	
8. Inoculated pelletted seed									
8h—hard pellets	100	99							
8s—soft pellets	99	100							

below the crown of the plant. In limed soil [(b)] the roots were very stunted and discoloured, although towards the tips they were often healthy, presumably because they had grown below the soil zone affected by the lime treatment. The nodules were numerous but minute, and although formed with effective rhizobia, they often showed no pink colouration. With both lime and micronutrients [(c)] the roots were healthy and vigorous; the nodules were numerous, and in the inoculated plots they were well formed and bright pink in colour. In the uninoculated plots the nodules were mainly ineffective, but some effective nodules occurred.

With *L. uliginosus* the uninoculated plants were well nodulated, and no response from inoculation could be expected. Nodulation was not so dependent upon the presence of lime as with the clovers, 46% or more of the plants being nodulated in the various unlimed treatments. Lime, however, did increase the percentage of nodulated plants, and micronutrients improved the size and colour of the nodules present.

L. corniculatus was slower in initial development and nodulation than the autotetraploid *L. uliginosus*. There was no significant effect from inoculation. Nodulation was very low without lime, but markedly increased in the presence of lime, although many of the nodules were small and white. Nodulation was further increased by the addition of micronutrients, and the size and colour of the nodules improved.

A few isolations were made from the infrequent effective nodules on the uninoculated subterranean clover plants in the lime plus micronutrients treatment; and two isolations were made from inoculated plants of this treatment. These isolates were tested

TABLE 4. Numbers of Viable Cells of Clover and Lotus Rhizobia per Gram

Date of Sampling	Description of Samples	Clover Rhizobia	Lotus Rhizobia
10.5.57	Undisturbed gumland	0	0
6.3.58	Cultivated unlimed soil where Experiments 1 and 2 later laid down	0	25
		0	0.7
		0	25
	Cultivated limed soil where field plots later sown	25	25
		0.7	25
8.5.58	Cultivated unlimed soil near Experiments 1 and 2	0	15
		0	35
		0	350
		0	3.5
	Between rows in uninoculated unlimed plot of Experiment 1	0	15
	Between rows in uninoculated limed plot on Experiment 1	35	700
26.6.58	Undisturbed gumland, wet spot	0	0
	Undisturbed gumland, dry spot	0	0

TABLE 5. Numbers of Viable Cells of Clover and Lotus Rhizobia per Gram in Soil Samples from Plots of Experiment 2

			Clover Rhizobia			Lotus Rhizobia		
	Date of Sampling	⟶	21.3.58	8.5.58	27.6.58	21.3.58	8.5.58	27.6.58
Plot	Fertiliser	Inoculation						
1	O	+	*	1.5	0	*	3,500	35
2	P, K	+		0	0		15	7,000
3	Ca	+			150,000			350,000
4	P, K, Ca	+	25,000	350,000	35,000	*	350,000	70,000
5	P, K, Ca, micros	+	*	1,500	700,000	7,000	15,000	1,500,000
6	P, K, Ca, micros	—	0	0.35	35	25	70	3,500
7	P, K, Ca	+	*	70,000	350,000	*	7,000	350,000

* Nodules present in all tubes at the dilutions used, numbers of rhizobia ≧ 700 in plot 1
≧ 70,000 in plots 4, 5, and 7

for effectiveness on subterranean, white, and red clover, and later were tested serologically against antisera for the inoculant strain and for the strains present in the commercial inoculant used in the field sowings. Out of six isolates, five were clearly distinct from the strain used for the inoculation, and the sixth was uncertain. One of these isolates was not distinguishable from a strain present in the commercial inoculant, and could possibly have arisen from this source. The two isolates taken from the inoculated plants were both indistinguishable from the inoculant strain.

Rhizobial Counts

The counts of clover and lotus rhizobia obtained from various soil samples are given in Table 4, and those from soil samples obtained from the plots of Experiment 2 in Table 5.

No clover or lotus rhizobia were detected in the three samples of undisturbed gumland soil. Cultivated but unlimed soil showed no clover rhizobia in any sample, but small numbers of lotus rhizobia were present in all samples. Both types of rhizobia were detected in the samples of limed soil. The clover rhizobia in the samples from the area later sown to field plots were predominantly ineffective on subterranean clover; those in the sample taken from between the rows in the limed uninoculated plot were effective.

In the plots of Experiment 2 without lime, the added clover rhizobia died out, but lotus rhizobia remained. In all plots with lime added, both clover and lotus rhizobia survived and probably multiplied. No further marked increase in numbers was obtained by the addition of fertilisers and micronutrients. The results from plot 7, which had been newly broken in from undeveloped gumland, showed that prior fallowing was not essential to obtain establishment of rhizobia. In plot 6, where no rhizobia were initially added, the numbers of clover and lotus rhizobia increased over the period, some of the clover rhizobia being ineffective on subterranean clover.

Clover rhizobia effective on subterranean clover were still present in the limed plots the following spring, as was shown by the effective nodulation on uninoculated subterranean clover sown in rows across the plots.

TABLE 6. Percentages of Nodulated Plants of the Three Clover Species from the Field Plots

Fertiliser	Subterranean Clover		White Clover		Red Clover	
	Inoculated	Not Inoculated	Inoculated	Not Inoculated	Inoculated	Not Inoculated
$L_{20}P_3K_0$	53.2±7.3	71.7±6.6	64.0±6.8	76.0±6.0	60.4±6.7	61.5±6.7
$L_{20}P_3K_0$ + B + Cu	71.2±6.3	76.0±6.0	57.4±6.7	78.6±5.5	62.7±6.8	60.4±6.7
$L_{00}P_9K_2$	93.6±3.6	91.1±4.2	98.1±1.9	98.1±1.9	92.2±3.8	95.6±2.8
$L_{00}P_9K_2$ + B + Cu	98.0±2.0	100	98.1±1.9	98.1±1.9	95.9±2.8	95.8±2.9

Nodulation of Seedlings taken from Field Sowings

The percentages of nodulated plants of the three clover species are given in Table 6.

In each species there was a highly significant increase in percentage of nodulated plants at the higher fertiliser rate. This increase was probably due to the higher rate of liming, since lime, as shown in Table 2, had a marked effect on nodulation of clover in this acid soil. Inoculation gave no significant increase in nodulation with any species, and boron plus copper gave no effect with white or red clover, but with subterranean clover the addition of these micronutrients gave an almost significant to significant increase at the two fertiliser rates. With white and red clover most of the nodules appeared effective, but with subterranean clover a high proportion of the nodules appeared to be ineffective. This is shown in Table 7.

TABLE 7. Percentage of Subterranean Clover Plants from the Field Plots with Apparently Effective Nodules (*)

Fertiliser	Apparently Effective Nodules		Apparently Effective Nodules near Crown †	
	Inoculated	Not Inoculated	Inoculated	Not Inoculated
$L_{20}P_3K_0$	10.6±4.5	28.3±6.6	0.0	2.2±2.2
$L_{20}P_3K_0$ + B + Cu	15.4±5.0	26.0±6.2	3.8±2.7	4.0±2.0
$L_{60}P_9K_2$	36.2±7.0	8.9±4.2	14.9±5.2	4.4±3.1
$L_{60}P_9K_2$ + B + Cu	33.3±6.6	22.0±5.9	11.8±4.5	4.0±2.0

* Nodules showing some pink colouration.
† Within about 1 inch of the crown.

Any effect from the inoculation should be most clearly shown by the effective nodules near the crown, since these would be the ones most likely to have arisen from the inoculant. Inoculation was found to give a significant increase in these nodules in the plots at the higher fertiliser rate but not in those at the lower rate, though even at the higher rate the proportion of plants carrying these nodules was very low.

The poor nodulation of the subterranean clover in the field plots was clearly shown by the subsequent development of the plants. As the available soil nitrogen was used up, the subterranean clover plants, in contrast with the white and red clover, became yellow and their growth became checked, with only a very few plants remaining green and growing vigorously. On 9 June, 25 of the larger, healthier plants were received for nodule examination, and only 10 were found to be carrying fully effective nodules. The others carried nodules typical of partial effectiveness—medium size to sometimes large and branched, but pale pink towards the tip only, and green or grey at the base.

Discussion

Although lotus plants grew abundantly in the swampy flats below the cultivated area, and clovers in developed pastures not far away, no host plants for either clover or lotus rhizobia were present in the scrubby vegetation growing on the undisturbed gumland soil. The only legume present was gorse. This was confined to the better-drained portions, which were of a different soil type, lacking the silica pan characteristic of Wharekohe silt loam. Considering the low pH—about 4.5—and infertility of the soil, it was not unexpected that clover and lotus rhizobia could not be detected in samples of the undisturbed soil. However, it was not anticipated that by the time the plots were put down this situation would have completely changed, and lotus rhizobia would be found to have become established, though in small numbers, apparently throughout the cultivated soil, and clover rhizobia also in the cultivated areas which had been limed.

From the few tests on undisturbed soil reported here it is not certain whether the rhizobia were in reality absent initially from the soil and were brought in on cultivating implements, on dust, or by other means, or whether they were initially present in the soil in occasional small pockets with a more favourable micro-environment, and were spread by the cultivations. This distinction becomes of importance where large blocks of land are being developed, as it is unlikely that an adequate spread of rhizobia, brought in by chance, would be obtained.

The following points are indicated by the rhizobial counts:

(1) The soil in the undisturbed state was not a favourable medium for clover or lotus rhizobia.

(2) Cultivation alone rendered the soil somewhat more favourable for lotus rhizobia, but it was still unfavourable for clover rhizobia.

(3) The addition of lime alone resulted in the soil becoming a relatively favourable medium for both clover and lotus rhizobia. Thus even in this extremely phosphate-deficient soil the addition of phosphate did not appear to be necessary for the rhizobia. However, owing to the low phosphate-fixing capacity of this soil (Wells and Saunders 1960) the very small amount of organic phosphorus mineralised as a result of cultivation would be relatively available.

The clover rhizobia that became established in the soil after liming were predominantly ineffective on subterranean clover. Although they were not detected in any samples of cultivated unlimed soil, they were either present in very small numbers in the soil used for Experiments 1 and 2, or were introduced by some means after the experiments were put down, as they appeared in plot 6 of Experiment 2, and in the nodules of subterranean clover in the limed uninoculated plots of Experiment 1.

A marked depression in growth, particularly with subterranean clover, was given by lime at the rate of application used in Experiment 1. This depression was greater than that obtained by Jackman (1961) in similarly treated plots nearby, possibly through the lime being mixed into a lesser depth of soil. The growth depression was apparently due to induced boron deficiency (Jackman 1961). Deficiency of boron is known to inhibit normal development of nodules (Brenchley and Thornton 1925; Mulder 1948). The addition of the micronutrient mixture actually increased the amount of nodulation, as well as markedly improving the form and colour of the nodules and the general growth of the plants. Also in the field trial the only effect noted from the addition of boron and copper was an effect on nodulation. A slight but significant increase in total nodulation was obtained with subterranean clover, which proved to be the most sensitive clover species to this micronutrient deficiency in these experiments.

The interpretation of the results in Experiment 1 is complicated by the presence of rhizobia in the soil, and by the micronutrient deficiency induced by the lime treatment. Nevertheless, it would appear that, provided reasonably generous initial fertilisation is given, plentiful early nodulation should be obtained without difficulty using inoculated clover or lotus seed sown on land of this soil type. Lime is essential for good early nodulation of the clovers and *L. corniculatus*, and beneficial for *L. uliginosus*. In unlimed soil inoculated lime-pelletted clover seed gave good nodulation and early growth. However, to maintain satisfactory clover growth it would be necessary to apply lime soon after sowing. Inoculated lime-pelletted seed could be of value in enabling a satisfactory stand of clover to be obtained where it was not possible to apply lime before sowing, and also where it was suspected that the lime had been applied unevenly, with pockets of unlimed soil.

In Experiment 1 inoculation proved not to be necessary with lotus, due to the build up of lotus rhizobia in the soil, but it is not known whether this build up can be relied on always to occur. With the clover, inoculation improved initial nodulation; however, it is probable that, with white clover, in the uninoculated plots of the lime plus micronutrients treatment, adequate nodulation would have developed later, owing to build up of clover rhizobia in the soil, the available nitrogen supply being adequate to sustain the plants until they nodulated. With subterranean clover, since most of the soil rhizobia were ineffective, inoculation was essential, and in Experiment 1 was successful. However, in the field plots the subterranean clover formed mainly ineffective nodules, even when inoculated. The reason for this inoculation failure is not clear. Conditions were different in several respects from those of the plot sowing. Rather lumpy, impure lime had been applied some time before sowing, allowing rhizobia ineffective on subterranean clover to multiply in the soil. The inoculant was different, and was applied dry to the seed, a method found satisfactory for roller-drilled seed on pumice soil (Sears *et al.* 1955), but possibly not so satisfactory for broadcast seed on this soil, followed as it was by

a rather dry period after sowing. Further investigations on these points are being made.

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INSECTS ASSOCIATED WITH THE MAJOR FODDER CROPS IN THE NORTH ISLAND

IV. Hymenoptera

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Summary

A survey during 1959 of the fodder crops in the North Island revealed some 73 species of Hymenoptera in association. Their identity, distribution, incidence, and possible economic importance are discussed.

Introduction

A survey of insects associated with fodder crops in the North Island was carried out during the summer of 1959. A general account of this survey, and the information in respect of the Hemiptera and Coleoptera have already been presented (Eyles 1960a, b, 1961). The present paper, which concerns the Hymenoptera collected in this survey, gives information on their distribution, incidence, and possible economic importance. The 129 samplings involved comprise chou moellier (24 crops), chou moellier plus swedes (19), lucerne (15), maize (33), mangels (4), pampas (1), rape (1), swedes (8), turnips (21), and mixed rape, swedes, and turnips (3).

Distribution maps for 17 species are presented, a black square indicating at least one occurrence within that area.

Further information on many of the species is contained in the account of Hymenoptera associated with pastures (Cumber 1959a), and is indicated by the placing of an asterisk (*) after the species name.

Results

Tenthredinoidea

Fam. Tenthredinidae (Saw-flies)

This family includes a great number of species, many of which are highly damaging to agriculture and forestry. Only a few introduced and endemic species occur in New Zealand, however.

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Hy. 105 *Caliroa limacina* de Geer

The larva of this species, commonly known as the "pear slug," is damaging to fruit tree foliage. A single stray adult was taken from a chou moellier crop at Waitoki on 2.3.59.

Ichneumonoidea

Fam. Ichneumonidae (Ichneumon Flies)

This extensive family parasitises several orders of insects but mainly the pre-adult stages of Lepidoptera. It includes many beneficial species.

Hy. 108 *Angitia cerophaga* (Grav.)

This species was taken in numbers up to nine from widely distributed North Island districts (Fig. 4). It occurred commonly in samples from chou moellier, rape, swedes, turnips, and once from maize.

Hy. 94 *Bassus laetatorius* F.

This European species occurs widely in both North and South Islands. The distribution encountered in the present survey is shown in Fig. 1. The species was taken in numbers up to six from chou moellier, lucerne, rape, swedes, and turnips during the months of February, March, and April. This species is known to parasitise the

Fig. 1

Fig. 2

Fig. 1.—Distribution map for the ichneumonid *Bassus laetatorius*. Hy. 94.

Fig. 2.—Distribution map for the ichneumonid *Cremastus* sp. Hy. 36A.

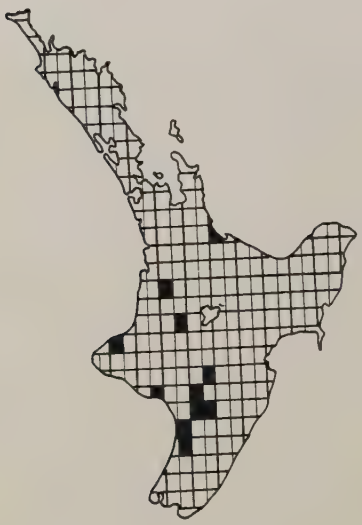

Fig. 3

Fig. 4

Fig. 3.—Distribution map for the ichneumonid *Horogenes* sp. Hy. 36.

Fig. 4.—Distribution map for the ichneumonid *Angitia cerophaga*. Hy.108.

larvae of syrphid flies. Gourlay (1930) records *Syrphus novae-zealandiae* Macq. as a host. It cannot be regarded as a beneficial parasite, however, since the larvae of many syrphid flies assist very materially in the control of aphid outbreaks.

Hy. 36A *Cremastus aginus* Kerrich*

This species is widely distributed in the North Island (Fig. 2). It was taken in numbers up to four from chou moellier, maize, swedes, and turnips during February, March, and April. Host relations are not known. Kerrich (1959) records allied species from psychid and phycitid pupae.

Hy. 96 *Diadromus collaris* (Grav.)

This species was taken in small numbers from turnips at Te Teko, 12.2.59, and Pukerua Bay, 25.3.59, and from mixed chou moellier and swedes, Maungaturoto, 26.2.59.

Hy. 100 *Echthromorpha intricatoria* Fabr.

This species is of widespread occurrence in Australia and New Zealand. In the present survey it was taken in numbers up to three from maize, Dargaville, 26.2.59; turnips, Marton, 24.3.59; chou moellier, Pahautanui, 25.3.59; and mixed chou moellier and swedes, Foxton and Levin, 25.3.59. Gourlay (1930) records the following pupal hosts: *Pyrameis gonerilla* Fabr., *Pyrameis itea* Fabr., *Mecyna*

maorialis Feld., *Nyctemera annulata* Boisd., *Oecticus omnivorous* Fered., and *Tyria jacobaeae* Linn. *Antheraea eucalypti* Sc., commonly known as the emperor gum moth, is also parasitised, but adults of the parasite appear unable to emerge from the tough cocoon. Conspicuous association of the adult parasites and larval populations of the noctuid species *Plusia chalcites* Esp. and *Heliothis armigera* Hubn. suggest that these are further hosts of this highly beneficial species.

Hy. 36 *Horogenes* sp.*

This species is widely distributed in areas of the North Island that are south of Auckland (Fig. 3). Small numbers were taken from chou moellier, lucerne, maize, swedes, and turnips during March and April. Host relations are not known.

Hy. 24 *Ichneumon promissorius* Erich.*

This species, which is also present in Australia, is of widespread occurrence in New Zealand. It was taken from turnips, Oruaiti, 23.2.59 and Otaki, 25.3.59, and from chou moellier, Pakaraka, 24.2.59 and Manakau, 25.3.59. Noctuid larvae are the probable hosts of this beneficial species.

Hy. 64 ? *Limnerium muelleri* Butl.*

Single specimens were taken from chou moellier plus swedes, Wanganui, 16.3.59, and from chou moellier, Marton, 24.3.59. The only

Fig. 5

Fig. 6

Fig. 5.—Distribution map for the braconid *Apanteles ruficrus*. Hy. 9.

Fig. 6.—Distribution map for the braconid *Trioxys* (*Aphidius*) *rapae*. Hy. 20.

recorded host is *Morova subfasciata* Walk., a thyridid which produces galls on *Muhlenbeckia*. It seems probable that other hosts exist.

Hy. 10 *Lissopimpla excelsa* (Costa)*

Single specimens were taken from mixed chou moellier and swedes, Mokau 16.3.59, and from chou moellier, Carterton–Masterton, 8.4.59. This is a beneficial species known to be parasitic on hepialid, geometrid, and noctuid larvae.

Hy. 116 *Xanthocryptus albopictus* (Sm.)

Two specimens were taken from chou moellier at Pahautanui on 25.3.59. Parrott (1953) and Gourlay (1930) indicate that this species is parasitic on the larvae of wood-boring Coleoptera (*Aemona* and *Xyloteles*).

Fig. 7

Fig. 8

Fig. 7.—Distribution map for the cynipid *Charips* (*Allotria*) *brassicae* Hy. 17A.

Fig. 8.—Distribution map for the figitid *Anacharis*? *zelandica*. Hy. 52.

Hy. 115 *Netelia ephippiatus* (Sm.)

Parrott (1951) indicates that this species is probably distributed throughout both Islands. In the present survey single specimens were taken from turnips, Makerua, 25.3.59, and swedes, Tokomaru, 25.3.59. It is recorded as a parasite of *Orthoclydon* (*Xanthorhoe*) *praefectata* (Walk.) but is also suspected of having noctuid hosts. It is a beneficial species.

Hy. 117 *Netelia productus* (Brulle)

Parrott (1951) indicates that this species occurs in Australia (including Tasmania) and is generally distributed throughout both Islands in New Zealand. A single specimen was taken from chou moellier, Manakau, 25.3.59. *Melanchra composita* Guen. and *Pseudaletia separata* (Walk.) are recorded by Gourlay (1930) as hosts of this beneficial species. *Netelia* spp. are mainly night-flying insects.

Hy. 92 Undetermined†

A single specimen was taken from turnips at Marumaru, 10.2.59.

Hy. 93 Undetermined

Single specimens were taken from turnips at Marumaru, 10.2.59, and from chou moellier, Bay View, 13.2.59.

Hy. 110 Undetermined

Single specimens were taken from maize at Pio Pio, 16.3.59, and from chou moellier, Kihikihi, 19.3.59 and Cheltenham, 24.3.59.

Hy. 112 Undetermined

A single specimen was taken from chou moellier, Taumarunui, 20.3.59.

Fig. 9

Fig. 10

Fig. 9.—Distribution map for the pteromalid near *Eupteromalus* sp. Hy 5.

Fig. 10.—Distribution map for the pteromalid *Pteromalus puparum*. Hy. 91.

† Undetermined species stand in the collection of the Entomology Division, Palmerston North, under the numbers shown.

Hy. 113 Undetermined

A single specimen was taken from turnips, Marton, 24.3.59.

Fam. Braconidae

These are mainly beneficial species, largely parasitic on larval Lepidoptera but also on aphids, scale insects, and Coleoptera.

Hy. 14 *Apanteles glomeratus* (L.)

Samples comprising up to six specimens were taken from mangels, Sanson, 24.3.59; chou moellier, Cheltenham, 24.3.59 and Pahautanui, 25.3.59; chou moellier plus swedes, Marton, 24.3.59 and Foxton, 25.3.59; chou moellier plus turnips, Greytown, 8.4.59.

Hy. 9 *Apanteles ruficrus* (Hal.)*

This cosmopolitan species occurred at widely distributed North Island localities (Fig. 5). Numbers up to five were taken from chou moellier, lucerne, maize, and mixed chou moellier and swedes. It occurred most commonly on maize; consequently one would suspect it to be parasitic on *Heliothis armigera* Hubn., which frequently damages the tassels and developing terminal grain. Parrott (1953) records this host in Australia. This highly beneficial species is known to attack *Plusia chalcites* Esp. in New Zealand.

Hy. 120 ? *Apanteles* sp.

A single specimen was taken from turnips at Parakao on 24.2.59.

Fig. 11.—Distribution map for the encyrtid ? *Ericydnus* sp. Hy. 38.

Fig. 12.—Distribution map for the encyrtid undetermined. Hy. 89.

Hy. 22 *Aspilota* sp.*

Single specimens were taken from lucerne, Cambridge, 3.3.59, and swedes, Pahiatua, 8.4.59.

Hy. 30 *Chorebus* sp.*

A single specimen was taken from rape at Feilding on 24.3.59.

Hy. 29 *Macrocentrus ? rubromaculatus* (Cam.)*

Single specimens were taken from lucerne at Takapau, 9.2.59; Waipawa, 9.2.59; and Te Awamutu, 19.3.59; from chou moellier at Manakau, 25.3.59; and maize, Shannon, 25.3.59.

Hy. 61 *Rogas* sp.*

This species was taken in small numbers from lucerne at Waiotapu, 12.2.59, and Bulls, 16.3.59.

Hy. 20 *Trioxys* (*Aphidius*) *rapae* (Curt.)*

This is a cosmopolitan species. In the present survey it was taken in numbers up to 16, during February, March, and April, from widely distributed areas in the North Island (Fig. 6). It also occurs widely in the South Island. This is the well known and highly beneficial parasite of the aphid *Brevicoryne brassicae* (L.). It was almost invariably present on cruciferous crops and also occasionally on non-crucifers, possibly being a stray or perhaps associated with cruciferous weeds.

Hy. 104 Undetermined

Two specimens were taken from turnips at Ruakaka on 2.3.59.

Hy. 107 Undetermined

A single specimen was taken from turnips at Rangariri on 3.3.59.

Cynipoidea

Fam. Cynipidae

The family includes gall formers, inquilines in galls, hyperparasites of aphids and coccids, and parasites of Diptera.

Hy. 17A *Charips* (*Allotria*) *brassicae* (Ashm.)*

This cosmopolitan species is widely distributed in the North Island (Fig. 7) and the South Island. It occurred commonly (February, March, and April) in numbers up to 65, on all cruciferous hosts, and occasionally in maize and lucerne sweepings, being stray or possibly associated with cruciferous weeds in the latter instances. In its role as a hyperparasite of *Brevicoryne brassicae* it cannot be regarded as a beneficial species since it is almost certainly parasitic on the highly useful aphid primary parasite *Trioxys rapae*.

Hy. 17 *Kleidotoma* sp.*

This species was taken in small numbers from maize, Torere, 12.2.59; turnips, Maungaturoto, 26.2.59; Otaki, 25.3.59; Masterton, 8.4.59; and mixed turnips and chou moellier, Greytown, 8.4.59. This species is probably parasitic on associated dipterous larvae.

Fam. Figitidae

The family includes parasites of Diptera and Neuroptera.

Hy. 52 *Anacharis* ? *zelandica* Ashm.*

This species was taken (February, March, and April) in most North Island districts (Fig. 8) in numbers up to five per sample. It occurred on chou moellier, lucerne, maize, swede, and turnip crops, and very frequently in association with *Micromus tasmaniae* Walk., strengthening the probability of its being parasitic on this hemerobiid. Such being the case it cannot be regarded as a beneficial species since the hemerobiid is a useful insect in that it is actively predacious on aphids.

Chalcidoidea

Fam. Pteromalidae

This is an extensive family and affects almost all orders of insects either as parasites or hyperparasites.

Fig. 13

Fig. 14

Fig. 13.—Distribution map for the eulophid *Chrysocharis* sp. H. 69.

Fig. 14.—Distribution map for the eulophid *Hemiptarsenus* sp. Hy. 3.

Hy. 5 Gen. near *Eupteromalus.**

This species was taken (February, March, and April) at localities throughout the North Island (Fig. 9). It occurred in numbers up to six on chou moellier, lucerne, maize, swedes, turnips, and mixtures including rape. It is probably parasitic on Diptera.

Hy. 91 *Pteromalus puparum* (Linn.)

This cosmopolitan species is well known as a valuable controlling agent of white butterflies (*Pieris* spp.). It was originally introduced into New Zealand in 1933, and is now widely distributed and contributing in no small measure to reduced *Pieris* populations. The distribution encountered in the present survey is shown in Fig. 10. Samples contained up to 82 specimens. Although its association was mainly with cruciferous crops occasional specimens were taken from maize, mangels, and lucerne. It was taken during February, March, and April.

Hy. 23 *Trichomalus* sp.*

Small numbers of this species were taken from chou moellier at Waipawa 9.2.59, Cambridge 3.3.59, Rukuhia 19.3.59, Palmerston North 24.3.59, and from maize at Whangarei 26.2.59.

Hy. 98 Undetermined

A single specimen was taken from maize at Kaitaia on 23.2.59.

Fam. Encyrtidae

This extensive family contains many beneficial species. They are mainly parasites of the ova, larvae, and pupae, parasitising many orders but more especially Hemiptera–Homoptera and Lepidoptera.

Hy. 38 *Ericydnus* sp.*

This species is widely distributed in the North Island (Fig. 11). It was taken on 32 occasions in numbers up to seven during February, March, and April. It occurred on all crops and without noticeable preferences. This species is known to parasitise *Sarcophaga milleri* J. et T. (Cumber 1960), having been reared on several occasions from puparia. It seems probable that several dipterous species are parasitised. The species is fairly commonly taken in pastures, the main host probably being *S. milleri*. Its presence in crops may be due to the shelter afforded, such crops usually being adjacent to pastures. Diptera breeding in the occasional rotting crucifer plants may possibly act as hosts, but there is no definite information on this point.

Hy. 89 Undetermined

This species is of widespread occurrence in the North Island (Fig. 12), being taken on 24 occasions during February, March, and April in numbers up to 12. No noticeable crop preferences are evident. Host relations are not known.

Fig. 15

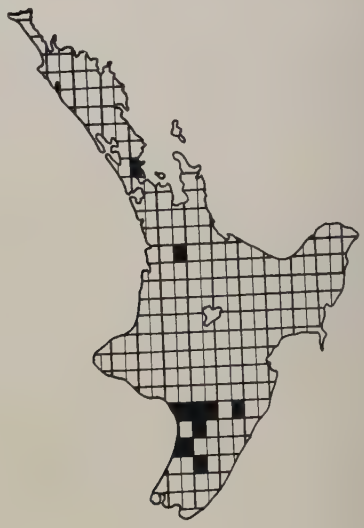

Fig. 16

Fig. 15.—Distribution map for the diapriid *Spilomicrus* sp. Hy. 19.

Fig. 16.—Distribution map for the ceraphronid *Lygocerus* sp. Hy. 57.

Hy. 90 Undetermined

Single specimens were taken from maize at Taradale, 9.2.59 and Ngawha, 25.2.59, and from mangels at Kimbolton on 24.3.59.

Hy. 95 Undetermined

A single specimen was taken from maize at Te Kaha on 11.2.59.

Hy. 111 Undetermined

A single specimen was taken from lucerne at Te Awamutu on 19.3.59.

Hy. 118 Undetermined

Single specimens were taken from lucerne at Takapau, 9.2.59, maize at Taradale, 9.2.59, and turnips at Tiniroto, 10.2.59.

Fam. Eulophidae

Most orders of insects are parasitised by members of this family, many species being highly beneficial biological control agents.

Hy. 35 *Aprostocetus* sp.*

A single specimen was taken from lucerne at Greenmeadows on 9.2.59.

Hy. 69 *Chrysocharis* sp.*

This species is of widespread occurrence in the North Island (Fig. 13), being taken on 17 occasions. It occurred on chou moellier, lucerne, maize, mangels, swedes, and turnips during February and March. It is possibly parasitic on *Phytomyza atricornis* Meig. (Gourlay 1930).

Hy. 21 *Closterocerus* sp.*

Single specimens were taken from maize at Kaitaia, 23.2.59, and Tauranga, 4.3.59, and from turnips at Ruakaka, 2.3.59, and mangels at Kimbolton, 24.3.59. It is, like the two species which follow, probably parasitic on Lepidoptera or leaf-mining Diptera.

Hy. 25 Gen. near *Dicladocerus**

A single specimen was taken from turnips at Otaki on 25.3.59.

Hy. 3 *Hemiptarsenus* sp.*

Small numbers of this species occurred at 15 localities (Fig. 14). It was taken during February, March, and April from chou moellier, lucerne, swedes and turnips.

Hy. 106 Undetermined

Three specimens were taken from maize at Helensville on 2.3.59.

Hy. 114 Undetermined

A single specimen occurred in a sample from chou moellier at Cheltenham on 24.3.59.

Hy. 119 Undetermined

A single specimen was taken from maize at Taradale on 9.2.59.

Fam. Elasmidae

Members of the family are parasitic on Diptera, Hemiptera, and Lepidoptera.

Hy. 34 *Elasmus* sp.*

A single specimen was taken from maize at Eureka on 3.3.59.

Fam. Mymaridae (Fairy Flies)

Representatives of the family are egg parasites, mainly on Hemiptera, but also of Coleoptera and Lepidoptera.

Hy. 81 Undetermined*

Single specimens were taken from lucerne at Kapiro on 24.2.59, maize at Ohinewai, 3.3.59, and mixed chou moellier and swedes at Otorohanga, 19.3.59.

Proctotrupoidea

Fam. Scelionidae

The members of this large family are egg parasites, mainly of Hemiptera, Lepidoptera, and Orthoptera, and include many beneficial species.

Hy. 1 *Baeus seminulum* Hal.*

A single specimen was taken from turnips at Parakao on 26.2.59.

Hy. 16 *Ceratoteleia* sp.*

Single specimens were taken from maize at Te Kaha, 11.2.59, and Kaitaia, 23.2.59, and from mixed maize and turnips at Maungaturoto, 26.2.59.

Hy. 2 *Microphanurus* sp.*

This species was taken in small numbers from maize at Wairoa, 10.2.59, Hangaroa, 10.2.59, Te Kaha, 11.2.59, Torere, 12.2.59, Ngawha, 25.2.59, and Awarua, 26.2.59, and from turnips at Waitoa, 3.3.59. This species will be parasitic on pentatomids.

Hy. 18 *Telenomus* sp.*

Two specimens were taken from lucerne at Te Awamutu on 19.3.59.

Hy. 31 Undetermined*

A single specimen was taken from maize at Whirinaki on 25.2.59.

Fig. 17.—Distribution map for the formicid *Ponera eduardi*. Hy. 37.

Hy. 46 Undetermined*

A single specimen was taken from maize at Helensville on 2.3.59.

Hy. 87 Undetermined*

A single specimen was taken from maize at Helensville on 2.3.59.

Hy. 97 Undetermined

A single specimen was taken from maize at Kaitaia on 23.2.59.

Hy. 103 Undetermined

A single specimen was taken from maize at Kamo on 2.3.59.

Fam. Platygasteridae

Most species in the family are parasitic on the larvae of Cecidomyidae.

Hy. 6A *Platygaster* sp.*

A single specimen was taken from maize at Eureka on 3.3.59.

Hy. 101 Undetermined

Single specimens were taken from maize at Dargaville, 25.2.59, and Helensville, 2.3.59, and lucerne at Bulls, 16.3.59.

Hy. 117 Undetermined

A single specimen was taken from lucerne at Takapau on 9.2.59.

Fam. Diapriidae

Members of the family are mainly parasites of Diptera.

Hy. 19 *Spilomicrus* sp.*

This species was taken in small numbers from crops of chou moellier, maize, mangels, swedes, and turnips on 17 occasions in widely separated North Island districts (Fig. 15). It occurred in February, March, and April.

Hy. 42 *Trichopria* sp.*

Small numbers were taken from lucerne at Takapau, 9.2.59, and Gisborne, 10.2.59, and from turnips at Marumaru, 10.2.59, and Tiniroto, 10.2.59.

Hy. 109 Undetermined

A single specimen was taken from swedes at Waitara on 16.3.59.

Fam. Ceraphronidae

Members of this family are often secondary parasites of aphids and coccids through braconid and chalcid primaries, and harmful in that they hinder the effects of beneficial primary species.

Hy. 7 *Aphanogmus* sp.*

Small numbers were taken from maize at Helensville, 2.3.59, and Rukuhia, 19.3.59, and from turnips at Pukerua Bay, 25.3.59.

Hy. 74 *Ceraphron* sp.*

Single specimens were taken from turnips at Tiniroto, 10.2.59, and Silverdale, 2.3.59, and from chou moellier at Whakatane, 12.2.59.

Hy. 57 *Lygocerus* sp.*

Only small numbers of this species were taken. Two occurrences were on lucerne, the remaining eight being on cruciferous crops (Fig. 16). The species was taken during February, March, and April.

Hy. 102 Undetermined

Single specimens were taken from mixed turnips and maize at Maungaturoto, 26.2.59, turnips at Parakao, 26.2.59, chou moellier at Pahautanui, 25.3.59, and maize at Ashhurst, 26.3.59.

Formicoidea

Fam. Formicidae

Several widespread and common species of ants have been taken during the survey.

Hy. 26 *Iridomyrmex glaber* (Mayr)*

Two workers were taken from lucerne at Kapiro on 24.2.59.

Hy. 37 *Ponera eduardi* Forel*

Males and an occasional queen were taken at 16 localities widely distributed in the North Island (Fig. 17), being present on most types of crop. The absence of workers suggests that they do not forage far above ground level, confirming the contention of Brown (1958) that the diet consists mainly of soil-inhabiting arthropods. Mating flights often take the form of drifting swarms, which would account for the catches when sweeping crops. Males were taken during February, March, and April, and queens in March and April.

Hy. 99 *Mesoponera castanea* (Mayr)

This species is known to be of widespread occurrence in the North Island (Cumber 1959b). A male was taken from maize at Whirinaki on 25.2.59.

Hy. 45 *Monomorium antarcticum* (White)*

This species is of widespread occurrence in both Islands. The present survey yielded the following: Takapau, lucerne, 9.2.59, 7 workers; Eureka, maize, 3.3.59, 1 male, 1 worker; Mahoenui, turnips, 16.3.59, 1 worker; Kihikihi, chou moellier, 19.3.59, 1 male; Te Kawa, maize, 19.3.59, 1 male; Marton, chou moellier, 24.3.59, 1 worker; Kimbolton, mangels, 24.3.59, 1 queen; and Greytown, mixed turnips and chou moellier, 8.4.59, 1 male.

The ant species encountered are not regarded as being of economic importance to crops.

Apoidea

Fam. Apidae

Hy. 55 *Apis mellifica Linn.* (Honey Bee)

This species was taken from lucerne at Greenmeadows, 9.2.59, and chou moellier at Cambridge, 3.3.59. The lucerne crop showed some flowering.

Discussion

The precise role played by each of the 73 species must remain unknown for some time to come. The majority of the species, however, are parasitic on other insects. Only when host relations are better known will it be possible to arrive at some conclusions as to the real importance of the various species. Some species, which have occurred in small numbers, may be casual visitors from adjacent areas and of no importance to the crop. But the presence of a species in small numbers does not necessarily mean that this is so, for potentially important pest species often balance with their parasites at relatively low population levels.

As the habits of each species become better known the associations and distributions shown by surveys such as the present one will aid very greatly in the understanding and control of the insect complex of fodder crops.

Acknowledgments

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APPENDIX

SPECIES OF HYMENOPTERA TAKEN FROM FODDER CROPS

	Lucerne	Maize	Chou Moellier	Chou Moellier, Swedes, Mixed	Swedes	Turnips	Rape	Mangels	Rape, Swedes, Turnips, Mixed
TENTHREDINIDAE									
Caliroa limacina			*						
ICHNEUMONIDAE									
Bassus laetatorius	*		*	*	*	*	*		
Cremastus agninus		*	*	*	*	*			
Echthromorpha intricatoria		*	*	*		*			
Horogenes sp. Hy. 36	*	*	*		*	*		*	
Angitia cerophaga Hy. 108		*	*	*	*	*	*		
Ichneumon promissorius			*			*			
Limnerium muelleri			*	*					
Lissopimpla excelsa			*	*					
Netelia ephippiatus					*	*			
Netelia productus			*						
Xanthocryptus albopictus			*						
Undetermined ichneumonid Hy. 92						*			
" " Hy. 93			*			*			
Diadromus collaris Hy. 96				*		*			
Undetermined ichneumonid Hy. 110		*	*	*					
" " Hy. 112			*						
" " Hy. 113						*			
BRACONIDAE									
Apanteles ruficrus	*	*	*	*					
Aspilota sp. Hy. 22	*				*				
Chorebus sp. Hy. 30							*		
Macrocentrus sp. Hy. 29	*	*	*						
Apanteles glomeratus Hy. 14			*	*	*	*		*	
Rogas sp. Hy. 61	*								
Trioxys rapae	*	*	*	*	*	*	*		*
Undetermined braconid Hy. 104						*			
" " Hy. 107						*			
CYNIPIDAE									
Charips brassicae	*	*	*	*	*	*	*		*
Kleidotoma sp. Hy. 17		*				*			
FIGITIDAE									
Anacharis sp. Hy. 52	*	*	*	*	*	*			
PTEROMALIDAE									
Near *Eupteromalus* sp. Hy. 5	*	*	*	*	*	*			*
Pteromalus puparum	*	*	*	*	*	*	*	*	*
Trichomalus sp. Hy. 23		*	*						
Undetermined pteromalid Hy. 98		*							
ENCYRTIDAE									
? *Ericydnus* sp. Hy. 38	*	*	*	*	*	*	*	*	
Undetermined encyrtid Hy. 89	*	*	*	*	*	*			
" " Hy. 90		*						*	
" " Hy. 95		*							

APPENDIX (CONTINUED)

	Lucerne	Maize	Chou Moellier	Chou Moellier, Swedes, Mixed	Swedes	Turnips	Rape	Mangels	Rape, Swedes, Turnips, Mixed
Undetermined encyrtid Hy. 111	*								
" " Hy. 118	*	*				*			
EULOPHIDAE									
Aprostocetus sp. Hy. 35	*								
Chrysocharis sp. Hy. 69	*	*	*	*	*	*		*	
Closterocerus sp. Hy. 21		*				*		*	
Near *Dicladocerus* sp. Hy. 25						*			
Hemiptarsenus sp. Hy. 3	*			*	*	*			
Undetermined eulophid Hy. 106		*							
Hy. 114			*	*					
" " Hy. 119		*							
ELASMIDAE									
Elasmus sp. Hy. 34		*							
MYMARIDAE									
Undetermined mymarid Hy. 81	*	*		*					
SCELIONIDAE									
Baeus seminulum						*			
Ceratoteleia sp. Hy. 16		*							
Microphanurus sp. Hy. 2		*				*			
Telenomus sp. Hy. 18	*								
Undetermined scelionid Hy. 31		*							
" " Hy. 46		*							
" " Hy. 87		*							
" " Hy. 97		*							
" " Hy. 103		*							
PLATYGASTERIDAE									
Platygaster sp. Hy. 6A		*							
Undetermined platygasterid Hy. 101	*	*							
" " Hy. 117	*								
DIAPRIIDAE									
Spilomicrus sp. Hy. 19	*	*		*	*	*		*	
Trichopria sp. Hy. 42	*					*			
Undetermined diaprid Hy. 109					*				
CERAPHRONIDAE									
Aphanogmus sp. Hy. 7		*				*			
Ceraphon sp. Hy. 74			*			*			*
Lygocerus sp. Hy. 57	*			*	*	*	*		
Undetermined ceraphronid Hy. 102		*	*			*			
FORMICIDAE									
Iridomyrmex glaber	*								
Ponera eduardi	*	*	*	*	*	*			
Mesoponera castanea		*							
Monomorium antarcticum	*	*	*		*	*		*	
APIDAE									
Apis mellifica	*		*						

INSECTS ASSOCIATED WITH THE MAJOR FODDER CROPS IN THE NORTH ISLAND

V. Diptera

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Summary

A survey during 1959 of the fodder crops in the North Island revealed some 50 species of Diptera in association. Their identity, distribution, incidence, and possible economic importance are discussed.

Introduction

A survey of insects associated with fodder crops in the North Island was carried out during the summer of 1959. A general account of this survey, and the information in respect of the Hemiptera, Coleoptera (Eyles 1960a, b, 1961), and Hymenoptera (Cumber and Eyles 1961) have already been presented. This paper, which concerns the Diptera collected in this survey, gives information on their distribution, incidence, and possible economic importance. The 129 samplings involved comprise chou moellier (24 crops), chou moellier plus swedes (19), lucerne (15), maize (33), mangels (4), pampas (1), rape (1), swedes (8), turnips (21) and mixed rape, swedes and turnips (3).

Distribution maps for 19 species are presented, a black square indicating at least one occurrence within that area.

Many species have not been positively identified, and this must await revisions of the families. This is particularly so in the Tipulidae, Chironomidae, Ceratopogonidae, and Cecidomyiidae.

Further information on many of the species is contained in the account of the Diptera associated with pastures (Cumber and Harrison 1959), and is indicated by the placing of an asterisk after the species name.

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Results

Sub-order **NEMATOCERA**

Fam. Tipulidae (Daddy-long-legs, Crane Flies)

There is a considerable New Zealand fauna, but little information on their biology. The family includes many species injurious in agriculture, the larval or "leather jacket" stages being easily recognised.

D. 15. Undetermined*†

This species was taken in 10 samples in numbers up to 6 (Fig. 1) from chou moellier, chou moellier mixed with swedes, maize, mangels and turnips. There was a preference for chou moellier and swedes but this may be due to its suitability as cover for a mainly day-resting species. It was taken in March and early April. The low numbers indicate the improbability of its general economic importance.

D. 59. Undetermined*

Single specimens were taken from turnips at Masterton and swedes at Pahiatua, both on 8.4.59.

D. 77. Undetermined

A single specimen was taken from lucerne at Taumarunui on 20.3.59.

Fig. 1.—Distribution map for the tipulid D. 15 undetermined.

Fig. 2.—Distribution map for the mycetophilid *Sciara agraria*. D. 11.

† Undetermined species are located in the collection of the Entomology Division, D.S.I.R., Palmerston North, under the numbers shown.

D. 85. Undetermined

A single specimen was taken from swedes at Pahiatua on 8.4.59.

Fam. Psychodidae (Moth-flies)

Insects in this family are small and fragile, with larval stages occurring in water, damp refuse, vegetation, etc.

D. 25. Undetermined

Small numbers were taken from maize at Kamo, 2.3.59; mangels at Tiakitahuna, 25.3.59; turnips at Makerua, 25.3.59; and swedes at Pahiatua, 8.4.59.

D. 80. Undetermined

A single specimen was taken from turnips at Ohingaiti, 20.3.59.

Fam. Culicidae (Mosquitoes)

Mosquitoes are well known the world over because of their nuisance and ability to transmit diseases.

D. 67. *Culex* sp.*

A single specimen was taken from mangels at Tiakitahuna on 25.3.59.

Fig. 3

Fig. 4

Fig. 3.—Distribution map for the mycetophilid *Sciara* sp. D. 54.

Fig. 4.—Distribution map for the chironomid D. 18 undetermined.

Fig. 5

Fig. 6

Fig. 5.—Distribution map for the dolichopodid *Chrysosoma mobile* D. 73.
Fig. 6.—Distribution map for the lonchopterid *Lonchoptera dubia* D. 6.

Fam. CECIDOMYIDAE (Gall Midges)

A revision of this family is much needed in New Zealand, for many species are of considerable economic importance.

D. 14. Undetermined*

Small numbers were taken from lucerne at Waipawa, 9.2.59, and Ohingaiti, 20.3.59; from maize at Whakatane, 12.2.59; and swedes at Pahiatua 8.4.59.

Fam. MYCETOPHYLIDAE (Fungus Gnats)

The majority of species depend upon fungus materials and organic matter for larval development, but a few root-damaging species do occur.

D. 56. *Mycetophila ? impunctata* Edw.*

Single specimens were taken from chou moelier at Palmerston North 24.3.59 and swedes at Makerua 26.3.59.

D. 11. *Sciara agraria* Felt*

This cosmopolitan species is of widespread occurrence in the North Island (Fig. 2). It was taken in numbers up to 23 from chou moellier, chou moellier plus swedes, lucerne, maize, mangels, swedes and turnips during the months of February, March, and April. Its economic importance cannot be gauged without closer studies.

Fig. 7

Fig. 8

Fig. 7.—Distribution map for the phorid ? *Megaselia* sp. D. 7.

Fig. 8.—Distribution map for the syrphid *Melanostoma fasciatum* D. 2.

D. 54. *Sciara* sp.*

This species occurred widely in the North Island (Fig. 3). It was taken in numbers up to 7 from chou moellier, chou moellier plus swedes, lucerne, maize, and turnips during February, March, and April.

Fam. Chironomidae (Midges)

The members of this family have aquatic larval stages and are of little economic importance. The adults disperse widely.

D. 17. Undetermined

Single specimens were taken from maize, Wairoa 10.2.59; lucerne, Cambridge 3.3.59; and swedes, Pahiatua 8.4.59.

D. 18. Undetermined*

This species occurred in 70 samples and is apparently present in most parts of the North Island (Fig. 4). It was taken (February till April) from all crops and without apparent preferences. Numbers in excess of 50 were sometimes taken.

D. 33. Undetermined*

Small numbers of this species were taken from maize at Waipawa 9.2.59, Te Kaha 11.2.59, and Ohinewai, 3.3.59, and from mixed chou moellier and swedes at Kimbolton 24.3.59.

D. 82. Undetermined

A single specimen was taken from turnips at Otaki on 25.3.59.

Sub-order **BRACHYCERA**

Fam. Stratiomyidae

Imms (1957) states: "the eggs are laid on plants near the edge of water, or even on the surface of the water, also in dung and in the soil. The larvae are carnivorous or saprophagous, and either terrestrial or aquatic, the terrestrial larvae being largely scavengers. . . ."

D. 69. *Eulalia atrovirens* (Bigot)

A single specimen was taken from lucerne at Takapau on 9.2.59.

D. 79. *Neoexaireta spiniger* Wied.

A single specimen was taken from turnips at Ohingaiti on 20.3.59. This species breeds readily in rotting vegetation.

Fam. Empididae

The larvae of empid species are found mainly in the soil, humus, rotting wood, etc. The adults are predacious and attack mainly Diptera.

D. 72. *Hilarempis minthaphila* Coll.

This species was taken from turnips at Tiniroto 10.2.59. (1 specimen) and swedes at Waimangu 12.2.59 (9).

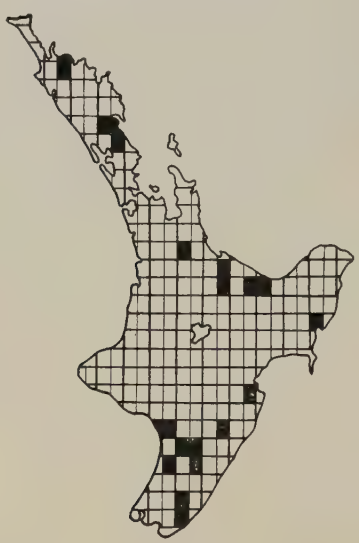

Fig. 9

Fig. 10

Fig. 9.—Distribution map for the agromyzid *Cerodontha denticornis* D. 24.

Fig. 10.—Distribution map for the sepsid *Lasionemapoda hirsuta* D. 5.

Fam. Dolichopodidae

The larvae in this family are mainly saprophagous and found under moist conditions. Adults are predacious on soft-bodied insects.

D. 73. *Chrysosoma mobile* Hutt.

This species is of widespread occurrence in mid-North Island districts (Fig. 5). It was taken during February and March in small numbers from crops of chou moellier, maize, swedes and turnips, and without noticeable preferences.

D. 76. Undetermined

Single specimens were taken from maize, Eureka 3.3.59; swedes, Ngaere 16.3.59; and turnips, Ohingaiti 20.3.59.

Sub-order **CYCLORRHAPHA**

Aschiza

Fam. Lonchopteridae

The larval stages in this family are found in decaying vegetable matter.

D. 6. *Lonchoptera dubia* Curran*

This cosmopolitan species occurred widely in the North Island (Fig. 6). It was taken without crop preference in numbers up to 24 from 42 localities during February, March, and April. It is not of economic importance.

Fig. 11

Fig. 12

Fig. 11.—Distribution map for the drosophilid *Scaptomyza fuscitarsis* D. 34.

Fig. 12.—Distribution map for the sphaerocerid *Leptocera thomasi* D. 20.

Fam. Phoridae

The larvae of the majority of species occur in decaying vegetable matter.

D. 7. ? *Megaselia* sp.*

This species was taken on 19 occasions during February, March, and April in widely distributed North Island districts (Fig. 7). It occurred in numbers up to 7 and without apparent crop preferences.

D. 13. Undetermined*

Small numbers were taken from turnips at Parakao 26.2.59, and from maize at Shannon 25.3.59.

Fam. Syrphidae (Hover Flies)

The larval habits in this family are extremely varied. Many predacious species are beneficial insects.

D. 46. *Eristalis tenax* L.*

This cosmopolitan species is of widespread occurrence in New Zealand. It is often confused with the honey-bee. Single specimens were taken from chou moellier at Pakaraka on 24.2.59 and turnips at Makerua on 25.3.59.

Fig. 13

Fig. 14

Fig. 13.—Distribution map for the ephydrid *Hydrellia tritici* D. 23.

Fig. 14.—Distribution map for the ephydrid *Psilopa metallica* D. 21.

D. 2. *Melanostoma fasciatum* Macq.*

This species is widely distributed in the North Island (Fig. 8). It was taken on 44 occasions in numbers up to 7, and from all types of crop except pampas. It occurred during February, March, and April. This insect is generally regarded as a beneficial species for the larvae are active predators on aphids, and few crops are entirely free from such pests.

Schizophora-acalypterae

Fam. Trypetidae (Fruit Flies)

The larvae in this family are phytophagous, and many species overseas are important pests of fruit.

D. 78. *Trypanea completa* Mall.

A single specimen was taken from lucerne at Taumarunui on 20.3.59.

Fam. Agromyzidae

The majority of species in this family have phytophagous mining larvae. Many are of economic importance.

D. 24. *Cerodontha denticornis* (Panz.)* (Wheat Sheath Miner)

This cosmopolitan species occurs throughout New Zealand. In the present survey it was taken on 24 occasions (Fig. 9) but only in small numbers. It occurred during February, March, and April. There are no apparent crop preferences. It must be regarded as a stray from adjacent or interspersed grasses.

D. 74. *Haplomyza chenopodii* Watt

This species was taken from turnips at Te Teko 12.2.59 (1 specimen), Ruakaka 2.3.59 (9), Te Kuiti 20.3.59 (1), Greytown 8.4.59 (1), and Masterton 8.4.59 (64), from chou moellier at Bulls 16.3.59 (2) and Pahautanui 25.3.59 (17), and from mixed chou moellier and swedes at Mokau 16.3.59 (1) and Kimbolton 24.3.59 (2). Harrison (1959) records this species from *Cerastium, Chenopodium, Beta, Stellaria,* and *Spinacia.*

D. 16. *Phytomyza atricornis* Meig.*

Single specimens were taken from turnips at Rangiriri and Waitoa on 3.3.59, maize at Tauranga 4.3.59, and 9 from chou moellier at Inglewood on 16.3.59. This is a cosmopolitan leaf-mining species with a large number of host plants.

Fam. Sepsidae

The larvae in this family are saprophagous and frequently associated with dung. They are of little economic importance.

Fig. 15

Fig. 16

Fig. 15.—Distribution map for the chloropid *Gaurax flavoapicalis* D. 70.
Fig. 16.—Distribution map for the chloropid *Gaurax mesopleuralis* D. 58.

D. 5. *Lasionemapoda hirsuta* de Meij.*

This cosmopolitan species was taken on 13 occasions (Fig. 10), which included 5 occurrences on maize and 4 on turnips. The maximum catch was 5. It was taken during February and March.

Fam. Sapromyzidae (Lauxaniidae)

In this family the larvae mainly feed on decaying vegetable matter, and are not of economic importance.

D. 36. *Poecilohetaerella bilineata* (Hutt.)*

Small numbers were taken from mixed chou moellier and swedes at Waimangu 12.2.59, lucerne at Waiotapu 12.2.59, and turnips at Te Puke 4.3.59.

Fam. Drosophilidae

Most species breed in decaying fruit, fermenting sap, etc., and are of little importance in agriculture.

D. 71. *Drosophila immigrans* Sturt.

Numbers of this species up to 14 were taken from turnips at Marumaru 10.2.59, Te Kuiti 20.3.59, Te Arakura 24.3.59, and maize at Te Kawa 19.3.59, and Dannevirke 26.3.59. Harrison (1959) records this species from Little Barrier Island to Nelson.

D. 34. *Scaptomyza fuscitarsis* Harr.*

This species was taken on 52 occasions and is present throughout the North Island (Fig. 11). It occurred on all types of crops studied, with the exception of pampas. The highest populations were taken from swedes, turnips, and maize, 320 specimens being present in one sample from swedes. Harrison (1959) records this species in association with rotting swedes. Its frequent occurrence in association with maize suggests that it may breed in moist materials held in the axils and sheaths. It was taken during February, March, and April.

Fam. Sphaeroceridae (Borboridae)

Organic matter and excrement are the main breeding sites for flies in this family, which is not important in agriculture.

D. 20. *Leptocera thomasi* Harr.*

This species was taken on 20 occasions in numbers up to 8 from widely distributed North Island districts (Fig. 12). It occurred from February till April, being taken from crops of chou moellier, lucerne, maize, swedes, and turnips, but mainly from crucifers.

Fam. Ephydridae

The species in this family are found in damp marshy meadows, etc., the larval stages being mainly phytophagous.

D. 8. *Hydrellia ? enderbii* (Hutt.)*

Four specimens were taken from a swede crop at Pahiatua on 8.4.59.

D. 23. *Hydrellia tritici* Coq.*

This is a cosmopolitan species, and its phytophagous larval habits with preference for the Gramineae are well known. In the present survey it occurred at widely distributed localities in the North Island (Fig. 13). No crop preferences were shown and the numbers were small, suggesting that it is a casual associate. It was taken from February till April.

D. 21. *Psilopa metallica* (Hutt.)*

This species was taken in numbers up to 13 from widely scattered localities south of Auckland (Fig. 14). No crop preferences are evident, and the numbers taken do not suggest that it is more than a casual associate. It occurred from February till April.

Fam. Chloropidae

The family contains many pests of economic crops.

D. 70. *Gaurax flavoapicalis* (Mall.)

Harrison (1959) gives the distribution of this species as Australia, and New Zealand from the Bay of Islands to Nelson.

In the present survey it was taken on 28 occasions (Fig. 15), the greatest catch being 90 from maize at Dannevirke on 26.3.59. The order of frequency of occurrence on crops was as follows: turnips, chou moellier plus swedes, swedes, maize and rape. This species was taken during February, March, and April. Harrison records this species swept from carrots, and bred from rotting swedes. This may well be a more damaging species than is generally recognised, and deserves investigation.

Fig. 17

Fig. 18

Fig. 17.—Distribution map for the chloropid *Oscinosoma badia* D. 12.
Fig. 18.—Distribution map for the calliphorid *Sarcophaga milleri* D. 1.

D. 58. *Gaurax mesopleuralis* (Beck.)*

Harrison (1959) records this species from New Zealand (Kaitaia, Bay of Islands, Auckland) for the first time and regards it as a relatively recent introduction from Australia. It is also recorded from Opotiki-Whakatane (Cumber and Harrison 1959). In the present survey it was taken on 41 occasions from widely distributed North Island districts (Fig. 16), the crops being maize (14), chou moellier (8), chou moellier plus swedes (6), turnips (8), lucerne (3), and swedes (2). The greatest catches (in excess of 40) were from turnips and maize. It appeared during February, March and April. This is possibly an injurious species, and deserves closer study.

D. 26. *Melanum neozelandicum* Mall.*

A single specimen was taken from swedes at Makerua on 25.3.59.

D. 12. *Oscinosoma badia* (Hutt.)*

This species is of widespread occurrence in the North Island (Fig. 17). Harrison (1959) reports it from Nelson to Queenstown in the South Island. In the present survey numbers up to 16 were taken, the crops including turnips, chou moellier, swedes, rape, lucerne and mangels, with an apparent preference for crucifers. The habits of the insect are not known. It was taken during February and March.

Fig. 19.—Distribution map for the muscid *Hylemyia deceptiva* D. 4.

Schizophora-calypterae

Fam. Calliphoridae

The larvae of flies belonging to this family may be saprophagous, flesh feeders, or parasites of arthropods.

D. 1. *Sarcophaga milleri* J. et T.*

This is one of the most common and widespread species in New Zealand. In the present survey nearly every crop swept produced specimens, catches of up to 40 being taken (Fig. 18). The highest catches were from cruciferous crops. The species breeds mainly in cattle droppings, but adults rest in longer vegetation, and feed on sweet secretions such as the sticky substances on paspalum heads. Adults were commonly observed on cruciferous crops feeding on the decomposing white butterfly larvae which had died from virus attack. This species is frequently confused with the biting stable fly *Stomoxys calcitrans* (L.) (Todd 1960a). It occurred from February till April.

D. 81. Undetermined

A single specimen was taken from mangels at Tiakitahuna on 25.3.59.

Fam. Tachinidae

Tachinid flies are parasitic, mainly on Coleoptera, Hemiptera, and Lepidoptera.

D. 47. Undetermined

A single specimen was taken from mangels at Kimbolton on 24.3.59.

D. 49. Undetermined

Two specimens were taken from turnips at Te Teko, and one from swedes at Waimangu, both on 12.2.59.

D. 84. Undetermined

A single specimen was taken from mangels at Ashhurst on 26.3.59.

Fam. Muscidae

The larval habits of species in this family are diverse. It includes important agricultural pests.

D. 3. *Hylemyia ? cana* (Macq.)*

Two specimens were taken from turnips at Te Puke on 4.3.59.

D. 4. *Hylemyia deceptiva* Mall.*

Small numbers were taken from chou moellier, maize, swedes, and turnips at widely distributed localities (Fig. 19), during February and March. The habits of this species are not known, but the numbers of specimens taken suggest that it is not of economic importance in crops.

Discussion

The majority of the crop types studied have close allies amongst vegetables, and since the scrutiny in private and market gardens is relatively intense, it is not surprising that the present survey has not revealed any recent introductions likely to be of particular importance. Nevertheless, there are several features worth consideration.

The syrphid *Melanostoma fasciatum* occurred quite commonly on cruciferous crops, and the inference is that it preys, in its larval stages, on the aphid species invariably present in some measure. Although this predator is not a complete answer in itself, its value should be taken into consideration in attempts to control aphids with sprays, so that control becomes an integrated programme. Basic studies are required before such may be carried out to best advantage.

Several species of chloropid flies, such as *Gaurax mesopleuralis*, *G. flavoapicalis*, and *Oscinosoma badia*, have turned up fairly consistently in crucifer and maize crops. Close studies may show that the larval stages are far more damaging than has hitherto been supposed, and that they are responsible in part for excess rotting of plant tissues.

The association of *Sarcophaga milleri* with putrefying virus-infected larvae of *Pieris rapae* on cruciferous crops has been mentioned. Such flies also are attracted to aphid-infested crops by the sticky honey-dew. It is possible that *S. milleri* assists in the spread of the virus, and is beneficial in this respect. The efficiency of this virus at certain times in the season is beyond doubt (Kelsey 1958, Todd 1960b). This fly is also closely associated with cattle and sheep droppings which are utilised by the larval stages, and thus could be of importance in the transmission of intestinal diseases.

Acknowledgments

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APPENDIX

SPECIES OF DIPTERA TAKEN FROM FODDER CROPS

	Lucerne	Maize	Chou Moellier	Chou Moellier, Swedes, Mixed	Swedes	Turnips	Rape	Mangels	Rape, Swedes, Turnips, Mixed
TIPULIDAE									
Undetermined tipulid D. 15		*	*	*		*		*	
" " D. 59					*	*			
" " D. 77	*								
" " D. 85					*				
PSYCHODIDAE									
Undetermined psychodid D. 25		*			*	*		*	
" " D. 80						*			
CULICIDAE									
Culex sp. D. 67								*	
CECIDOMYIDAE									
Undetermined cecidomyid D. 14	*	*			*				
MYCETOPHYLIDAE									
Sciara agraria	*	*	*	*	*	*		*	
Sciara sp. D. 54	*	*	*	*		*			
Mycetophila ? impunctata D. 56			*		*				
CHIRONOMIDAE									
Undetermined chironomid D. 17	*	*			*				
" " D. 18	*	*	*	*	*	*	*	*	
" " D. 33		*		*					
" " D. 82						*			
STRATIOMYIDAE									
Eulalia atrovirens	*								
Neoexaireta spiniger						*			
EMPIDIDAE									
Hilarempis minthaphila				*		*			
DOLICHOPODIDAE									
Chrysosoma mobile		*	*	*	*	*			
Undetermined dolichopodid D. 76		*			*	*			
LONCHOPTERIDAE									
Lonchoptera dubia	*	*	*	*	*	*		*	
PHORIDAE									
? Megaselia sp. D. 7	*	*	*	*	*	*			
Undetermined phorid D. 13		*				*			
SYRPHIDAE									
Eristalis tenax			*			*			
Melanostoma fasciatum	*	*	*	*	*	*	*	*	*
TRYPETIDAE									
Trypanea ? completa D. 78	*								

APPENDIX (Continued)

	Lucerne	Maize	Chou Moellier	Chou Moellier, Swedes, Mixed	Swedes	Turnips	Rape	Mangels	Rape, Swedes, Turnips, Mixed
AGROMYZIDAE									
Cerodontha denticornis	*	*	*	*	*	*			
Phytomyza atricornis		*	*			*			
Haplomyza chenopodii			*	*		*			
SEPSIDAE									
Lasionemapoda hirsuta	*	*		*	*	*			
SAPROMYZIDAE									
Poecilohetaerella bilineata	*			*		*			
DROSOPHILIDAE									
Scaptomyza fuscitarsis	*	*	*	*	*	*		*	
Drosophila immigrans		*				*			
SPHAEROCERIDAE									
Leptocera ? thomasi D. 20	*	*	*	*	*	*			
EPHYDRIDAE									
Hydrellia enderbii D. 8					*				
Hydrellia tritici	*	*	*	*	*	*		*	
Psilopa metallica	*	*	*	*	*	*		*	
CHLOROPIDAE									
Gaurax flavoapicalis		*	*	*	*	*	*		
Gaurax mesopleuralis	*	*	*	*	*	*			
Melanum neozelandicum					*				
Oscinosoma badia	*		*	*	*	*	*	*	
CALLIPHORIDAE									
Sarcophaga milleri	*	*	*	*	*	*	*	*	*
Undetermined calliphorid D. 81								*	
TACHINIDAE									
Undetermined tachinid D. 47				*		*		*	
" " D. 49								*	
" " D. 84									
MUSCIDAE									
Hylemyia ? cana D. 3						*			
Hylemyia deceptiva		*	*		*	*			

INSECTS ASSOCIATED WITH THE MAJOR FODDER CROPS IN THE NORTH ISLAND

VI. Odonata, Orthoptera, Isoptera, Psocoptera, Thysanoptera, Neuroptera, Lepidoptera

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Summary

A survey during 1959 of the fodder crops in the North Island revealed Odonata-1 species, Orthoptera-7, Isoptera-1, Psocoptera-3, Thysanoptera-11, Neuroptera-1, and Lepidoptera-14, in association. Their identity, distribution, incidence, and possible economic importance are discussed.

Introduction

A survey of insects associated with fodder crops in the North Island was carried out during the summer of 1959. A general account of this survey, and the information in respect of the Hemiptera and Coleoptera (Eyles 1960a, b, 1961), and Hymenoptera and Diptera (Cumber and Eyles 1961a, b), have already been presented. The present paper, which concerns the Odonata, Orthoptera, Isoptera, Psocoptera, Thysanoptera, Neuroptera, and Lepidoptera collected in this survey, gives information on their distribution, incidence, and possible economic importance. The 129 samplings involved comprise chou moellier (24 crops), chou moellier plus swedes (19), lucerne (15), maize (33), mangels (4), pampas (1), rape (1), swedes (8), turnips (21), and mixed rape, swedes and turnips (3).

Distribution maps for 11 species are presented, a black square indicating at least one occurrence within that area.

A number of species have not been positively identified, and others are obviously casually associated. Further information on many species is contained in the papers on insects associated with pastures (Cumber 1959a, b, c, 1960a, b), and is indicated by the placing of an asterisk after the species name.

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Results

Order **ODONATA** (Dragonflies)

The members of this order are predacious species, both as larvae and adults, the larvae being aquatic. The adults capture prey while on the wing, Diptera often being taken. They are regarded as beneficial in some areas because of their capacity to assist in the control of mosquito larvae and adults.

Fam. Lestidae

This is a world-wide family.

Od. 1. *Austrolestes colensonis* Wh.

Single specimens were taken from mixed chou moellier and swedes, Pio Pio 16.3.59; turnips, Te Arakura 24.3.59, and Otaki 25.3.59; mangels, Tiakitahuna 25.3.59; and maize, Dargaville 26.2.59. It is unlikely to be of economic importance in crops.

Order **ORTHOPTERA** (Grasshoppers, Locusts, Crickets, etc.)

Fam. Gryllidae (Crickets)

Or. 6. *Acheta commodus* (Walk.)* (The Common Field Cricket)

This species, well known for its depredations during dry seasons favouring cracked turf, etc., is nocturnal for the most part, and was not taken during sweep-netting. Soil examinations showed

Fig. 1

Fig. 2

Fig. 1.—Distribution map for the tettigoniid *Xiphidium semivittatum* Or. 1.
Fig. 2.—Distribution map for the psocid *Ectopsocus congener* P. 4.

its presence on several occasions. It was taken from under mixed chou moellier and swedes at Maungaturoto 26.2.59 (considerable numbers) and Levin 25.3.59; turnips, Maungaturoto (considerable numbers) 26.2.59; and maize, Kaitaia 23.2.59, Mangamuka 23.2.59, Dargaville 25.2.59. It is considered to be of only minor importance in fodder crops.

Or. 3. *Cyrtoxiphus* sp.*

A single adult was taken from turnips at Ruakaka on 2.3.59.

Or. 2. *Lissotrachelus maoricus* (Walk.)*

This native species is of widespread occurrence. In the present survey small numbers were taken from turnips, Takapau 9.2.59; lucerne, Waiotapu 12.2.59, Greytown 8.4.59; and maize, Pakaraka 24.2.59, and Kamo 2.3.59. It is not considered of economic importance.

Fam. Tettigoniidae (Long-horned Grasshoppers)

Or. 4. *Caedicia olivacea* Brunn.

This is the common katydid which is of widespread occurrence in New Zealand and Australia. It is on occasion damaging to shrubs, including young citrus, but is not regarded as being of economic importance in fodder crops. Single specimens were taken from maize at Hangaroa 10.2.59 (nymph), Awarua 26.2.59 (adult), and mixed chou moellier and swedes at Levin 25.3.59 (adult).

Or. 1. *Xiphidium semivittatum* Walk.*

This species, which is also present in Australia, is of widespread occurrence in both Islands of New Zealand, where it shows a preference for long grassy vegetation. In the present survey it was taken from chou moellier, lucerne, maize, mangels, and turnips in numbers up to 10, and from widely distributed districts (Fig. 1). Nymphs were taken during February and early March, adults from mid-February to early March. This species showed a decided preference for maize, and was observed on many occasions feeding on the "tassels", often taking them down below the level of the cob sheath and simulating the damage made by *Heliothis* larvae. The effects of this pest are not very great where maize is grown as green feed, but grain production could be seriously affected.

Fam. Acrididae (Short-horned Grasshoppers)

Or. 5. *Phaulacridium marginale* Walk.*

A single specimen was taken from lucerne at Martinborough on 8.4.59. Lowe (1956) records severe damage to turnips, rape, and lucerne near the Rangitata River. Early accounts indicate that Orthoptera generally may have been far more plentiful before the introduction of European birds.

Or. 7. *Locusta migratoria* L.*

Adults of this species were observed amongst turnips at Oruaiti on 23.2.59, and Silverdale, 2.3.59. Only small numbers were present.

Order **ISOPTERA** (Termites)

Fam. Kalotermitidae

I. 1. *Kalotermes brouni* (Frogg.)

This is a native species which occurs widely in New Zealand. A single specimen was taken from turnips at Tiniroto on 10.2.59. Winged adults disperse widely when seasonal conditions are right. Colonies live in dry wood.

Order **PSOCOPTERA** (Booklice or Psocids)

Most foliage will yield psocids at some time of the year. Here they shelter and feed upon algae, lichens, fungi, and fragments of animal and vegetable matter. They cannot be regarded as being of economic importance under such circumstances except perhaps in their possible capacity as fungus distributors.

Fam. Caeciliidae

P. 1. *Caecilius flavistigma* Tilly.*

Single specimens were taken from maize at Taradale 9.2.59, Kaitaia 23.2.59; lucerne at Bulls 16.3.59, Longburn 24.3.59; turnips at Mahoenui 16.3.59.

P. 4. *Ectopsocus congener* Tilly.*

This species was taken in numbers up to 12, from crops of maize, chou moellier, swedes, turnips, and mangels at widely distributed localities in the North Island (Fig. 2). It occurred most commonly on maize.

Fam. Myopsocidae

P. 5. *Myopsocus* sp.*

Two specimens of this unrecorded species were taken from mangels at Sanson on 24.3.59.

Order **THYSANOPTERA** (Thrips)

Sub-Order **TEREBRANTIA**

Fam. Aeolothripidae

T. 4. *Aeolothrips fasciatus* (L.)*

This cosmopolitan species is of widespread occurrence in both Islands. In the present survey it was taken in numbers up to 50 from crops of lucerne, maize, and chou moellier, with an apparent preference for lucerne (Fig. 3). It was taken in February, March and April. This species is predacious on small soft-bodied insects and is regarded as beneficial.

Fig. 3

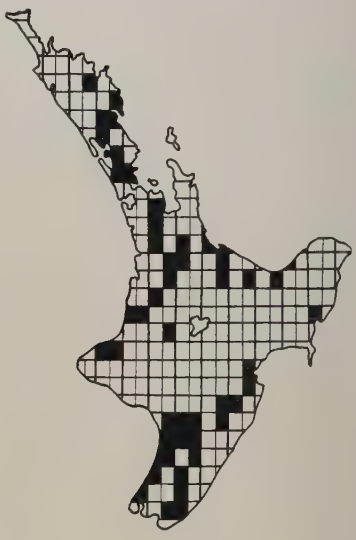

Fig. 4

Fig. 3.—Distribution map for the aeolothripid *Aeolothrips fasciatus* T. 4.
Fig. 4.—Distribution map for the thripid *Thrips tabaci* T. 7.

Fam. THRIPIDAE

T. 9. ? *Anaphothrips obscurus* Muller*

This species, which is of widespread occurrence in New Zealand, was taken in small numbers from chou moellier at Bulls 16.3.59, Urenui 16.3.59; lucerne at Waiotapu 12.2.59, Kapiro 24.3.59; maize at Te Kaha 11.2.59, Whakatane 12.2.59, Kaitaia 23.2.59, Ngawha 25.2.59, mixed chou moellier and swedes at Otorohanga 19.3.59; and turnips at Ruakaka 2.3.59, and Mahoenui 16.3.59. It is thought to be of little importance in crops.

T. 8. *Anaphothrips secticornis* Tyrbom.*

Small numbers were taken from lucerne at Waiotapu 12.2.59, Kapiro 24.2.59, Ohingaiti 20.3.59, Martinborough 8.4.59, and from maize at Ngawha 25.2.59, and Eureka 3.3.59.

T. 10. ? *Aptinothrips rufus* Gmelin*

Small numbers of this cosmopolitan species were taken from maize at Shannon 25.3.59, and mixed turnips and chou moellier at Greytown 8.4.59.

T. 5. *Heliothrips haemorrhoidalis* Bouche*

Small numbers of this cosmopolitan species, which is commonly known as the "greenhouse thrips", were taken from mixed chou

moellier and swede crops at Wharepaina 4.3.59, and Otorohanga 19.3.59.

T. 11. ? *Isoneurothrips obscuratus* Crawford*

This species, which has been recorded from a considerable number of host plants in both North and South Islands, was taken in small numbers from chou moellier at Waipukurau 9.2.59, Whakatane 12.2.59, Kihikihi 19.3.59; maize at Waipawa, 9.2.59, Hangaroa 10.2.59, Wairoa 10.2.59, Whakatane 12.2.59; and turnips, Ohingaiti 20.3.59.

T. 7. *Thrips tabaci* Lindeman*

This cosmopolitan species, commonly known as the "onion thrips," is of widespread occurrence in New Zealand. In the present survey it was taken, usually in small numbers, on 66 occasions, and from all types of crop studied (Fig. 4) It occurred in February, March, and April.

T. 13. ? *Thrips* sp.

Small numbers of this undetermined species were taken from chou moellier at Cheltenham 24.3.59, Pahautanui 25.3.59; maize at Maungaturoto 26.2.59, Eureka 3.3.59, Shannon 25.3.59; swedes at Pirinoa 8.4.59; and turnips, Pukerua Bay 25.3.59, Makerua 25.3.59.

Sub-Order **TUBULIFERA**

Fam. Phloeothripidae

T. 1. *Haplothrips niger* Osborne*

Small numbers of this cosmopolitan species were taken from lucerne at Waiotapu 12.2.59, and Martinborough 8.4.59; and turnips at Greytown 8.4.59.

T. 2. ? *Haplothrips* sp.*

Small numbers occurred on lucerne at Waipawa 9.2.59, and Martinborough 8.4.59.

T. 3. ? *Haplothrips* sp.*

A single specimen was taken from turnips at Ruakaka on 2.3.59.

Order **NEUROPTERA** (Alder Flies, Lacewings, Ant Lions)

Fam. Hemerobiidae

N. 1. *Micromus tasmaniae* Walk.*

This species, which is shared with Australia, is widely distributed in the North Island of New Zealand. In the present survey it was taken (February, March, April) in small numbers from crops of chou moellier, lucerne, maize, swedes and turnips (Fig. 5). It is a predacious and beneficial species, feeding upon aphids and scales.

Fig. 5

Fig. 6

Fig. 5.—Distribution map for the hemerobiid *Micromus tasmaniae* N. 1.
Fig. 6.—Distribution map for the tinaeid *Choreutis bjerkandrella* Le. 9.

Order **LEPIDOPTERA** (Butterflies, Moths)

Sub-Order **DITRYSIA**

Fam. COLEOPHORIDAE

Le. 1. *Coleophora alcyonipenella* Kol.* (Clover Case Bearer)

A single specimen was taken from a lucerne crop at Gisborne on 10.2.59.

Fam. PLUTELLIDAE

Le. 28. *Plutella maculipennis* Curt.

This is the cosmopolitan and well known "diamond-back moth" which may be very troublesome in cruciferous crops. It was taken as larvae, pupae, and adults from chou moellier at Whakatane 12.2.59, Urenui 16.3.59, Foxton 25.3.59, and from mixed chou moellier and swedes at Wanganui 16.3.59, Mokau 16.3.59, Mahoenui 16.3.59, Te Rauamoa 19.3.59. In some areas, notably at Urenui, the larvae were severely affected by an entomogenous fungus.

Fam. TINAEIDAE

Le. 9. *Choreutis bjerkandrella* Thunb *

This cosmopolitan species, which is widely distributed in the North Island, was taken from chou moellier, lucerne, maize, rape, swedes, and turnips during February, March, and April (Fig. 6). Only small numbers were taken; consequently, it was probably breeding on attendant composite weeds.

Le. 10. Undetermined*†

Small numbers were taken from maize at Eureka 3.3.59, and turnips at Te Teko 12.2.59 and Parakao 26.2.59.

Le. 12. Undetermined*

Numbers up to 8 of this species were taken from chou moellier at Inglewood 16.3.59, Urenui 16.3.59, Dannevirke 26.3.59, Martinborough, Greytown 8.4.59; lucerne at Takapau 9.2.59; mangels at Kimbolton 24.3.59; turnips at Pukerua Bay 25.3.59, Otaki 25.3.59, Makerua 25.3.59; mixed chou moellier and swedes at Mokau 16.3.59, and Pirinoa 8.4.59.

Le. 25. Undetermined

Two specimens were taken from lucerne at Cambridge on 3.3.59.

Fam. Tortricidae

Le. 26. *Austrotortrix postvittana* (Walk.)

This species, which also occurs in Australia, is widespread in New Zealand. It attacks a considerable number of plants. Information on its biology and parasites is given by Dumbleton (1932, 1940). In the present survey small numbers of adults and larvae were taken from chou moellier at Waitoki 2.3.59, Cambridge 3.3. 59, Kihikihi 19.3.59; lucerne at Bulls 16.3.59, Ohingaiti 20.3.59; mangels at Sanson 24.3.59; maize at Shannon 25.3.59; mixed chou moellier and swedes at Wanganui, Mokau 16.3.59.

Fam. Crambidae

Le. 4. *Crambus flexuosellus* Dbld.*

This species of "grass moth" is common from Auckland southwards. In the present survey it was taken in numbers up to 7 from chou moellier, lucerne, maize, mangels, rape, turnips, and chou moellier mixed with swedes, during February, March, and April (Fig. 7). There are no apparent host preferences. These moths fly at night and rest under foliage during the day. Larvae tunnel in silken galleries in the soil, and are probably associated with uncultivated areas.

Fam. Lycaenidae (Blues, Coppers)

Le. 3. *Lycaena labradus* (Godt.)*

This species is widespread in Indo-Pacific regions, and occurs throughout New Zealand. In the present survey it was taken in numbers up to 7 from crops of chou moellier, lucerne, maize, swedes, and turnips (Fig. 8). It is to be regarded as a casual associate, and doubtless breeds in relatively undisturbed areas of roughage. It was taken in February, March, and April (adults).

† Undetermined species are held under the numbers shown in the collections of the Entomology Division substation, D.S.I.R., Palmerston North.

Fig. 7

Fig. 8

Fig. 7.—Distribution map for the crambid *Crambus flexuosellus* Le. 4.
Fig. 8.—Distribution map for the lycaenid *Lycaena labradus* Le. 3.

Fig. 9

Fig. 10

Fig. 9.—Distribution map for the pierid *Pieris rapae* Le. 17.
Fig. 10.—Distribution map for the noctuid *Heliothis armigera* Le. 13.

Fam. Pieridae (Whites, etc.)

Le. 17. *Pieris rapae* (L.)*

This is the common "white butterfly," which is present throughout New Zealand. In the present survey it was taken either as adults or larvae on a great number of occasions, and mainly in association with cruciferous crops (Fig. 9). As many as 22 larvae were taken in some sweeps. On many occasions, despite moderate populations of adults over the crops and evidence of former larval damage, few active larvae could be found. This was mainly due to the virus disease (Kelsey 1958, Todd 1960).

Adults and larvae were present from February till April (duration of survey).

Fam. Geometridae (Carpets, Waves, Loopers, etc.)

Le. 5. *Xanthorhoe ? aegrota* (Butl.)*

Numbers up to 8 were taken from mixed chou moellier and swedes, Mokau 16.3.59; lucerne, Kapiro 24.2.59; maize, Pakaraka 24.2.59; and turnips, Te Teko 12.2.59, Rangiriri 3.3.59. Numbers taken do not suggest that it is a damaging species.

Fam. Arctiidae (Tiger Moths, etc.)

Le. 24. *Nyctemera annulata* (Boisd.)

This is the common and widespread "magpie moth," which is a native of this country and breeds on composites such as *Cineraria*, groundsel, ragwort, etc.

Fig. 11.—Distribution map for the noctuid *Pseudaletia separata* Le. 11.

A single adult was taken from mixed chou moellier and swedes at Maungaturoto 26.2.59.

Fam. Noctuidae (Cutworms, Armyworms, etc.)

Le. 22. *Agrotis ypsilon* Rott.*

This species, known more commonly as the "greasy cutworm," is cosmopolitan, and widely distributed in New Zealand. In the present survey it was taken only once as larvae in small numbers from mixed chou moellier and swedes at Maungaturoto 26.2.59.

Le. 13. *Heliothis armigera* Hubn.*

This cosmopolitan species is commonly known as the "tomato fruitworm", or the "corn earworm", and is a damaging pest of a great number of plants. In the present survey small numbers of adults were taken on 5 occasions, but larvae appeared 50 times (Fig. 10). The tassel damage in maize crops was often very evident. There may on occasions be some confusion here with the damage caused by the tettigoniid *Xiphidium semivittatum*. The latter species, however, does not eat down very far below the level of sheathing portions. This species (*H. armigera*) was by no means confined to maize in the present

Fig. 12.—Damage to maize foliage caused by the introduced armyworm *Pseudaletia separata* at Te Kawa, 19.3.59. Only the midribs of some leaves remain.

survey, however, for numbers of larvae approaching 30 were taken from cruciferous crops and lucerne, the importance of the damage here being difficult to assess without further close studies.

Le. 27. *Plusia chalcites* (Esp., Schmett.)

This is a cosmopolitan species, commonly known as the "silver Y moth," or the "looper caterpillar". It is not a geometrid, as the latter name would lead one to suspect. It attacks a wide variety of plants, including solanaceous species and beans. In the present survey it was taken in small numbers from chou moellier at Palmerston North 24.3.59; lucerne, Longburn 24.3.59; maize, Longburn 24.3.59; and turnips, Otaki 25.3.59. It is not considered to be an important pest of the crops studied.

Le. 11. *Pseudaletia separata* (Walk.)*

This species is better known as the "introduced armyworm," and is the one mainly concerned in North Island outbreaks. It is also present in the South Island. In the present survey it occurred mainly as larvae in crops of chou moellier mixed with swedes, lucerne, maize, and turnips, with a preference for maize (Figs. 11, 12). Under certain local conditions this species may be very damaging to pastures and grain crops. Detailed information on this and other noctuid species is given by Atkinson *et al.* (1956).

Discussion

This account concerns seven orders of insects found in association with fodder crops in the North Island. As indicated, many of the species encountered are casual associates only. No new unsuspected and obviously damaging species have been found.

Where it is customary to leave considerable adjacent areas of roughage, tassel damage by the tettigoniid *Xiphidium semivittatum* may be anticipated, and this in conjunction with attack by larvae of *Heliothis armigera* could well be responsible for reduced grain yields in maize. The real importance of the species of Thysanoptera encountered can only be determined by closer studies. The present investigation is essentially a study of the established crop rather than of the crop during establishment—the latter being a time when it is far more susceptible to the effects of small selective feeding insects. The hemerobiid *Micromus tasmaniae*, though not found in large numbers, must be considered as a beneficial insect, especially under conditions of aphid build-up.

The incidence of lepidopterous pests in fodder crops is much as was anticipated. There are two features, however, which deserve some consideration. The first is the high incidence of infection of larvae of *Pieris* by a putrefying virus. When this is active either the viability of new laid eggs is affected or larvae die soon after emergence. The second is the high incidence of an entomogenous fungus in larvae of *Plutella* in the Taranaki district. This, like the virus in *Pieris*, results

in a very real and advantageous control of the pest. It seems highly probable that a "seeding" of cruciferous crops with aqueous suspensions of these agents early in the season would minimise damage at this stage, and hurry the advantages which now appear naturally at a later date. Experimental work to devise suitable techniques would be well worth while.

Acknowledgments

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APPENDIX

Miscellaneous Species of Insects Taken from Fodder Crops

	Lucerne	Maize	Chou Moellier	Chou Moellier, Swedes, Mixed	Swedes	Turnips	Rape	Mangels	Rape, Swedes, Turnips, Mixed
ORDER ODONATA									
LESTIDAE									
Austrolestes colensonis		*		*		*		*	
ORDER ORTHOPTERA									
GRYLLIDAE									
Acheta commodus		*		*		*			
Cyrtoxiphus sp. Or. 3						*			
Lissotrachelus maoricus	*	*				*			
TETTIGONIIDAE									
Caedicia olivacea		*		*					
Xiphidium semivittatum	*	*	*			*		*	
ACRIDIDAE									
Phaulacridium marginale	*								
Locusta migratoria						*			
ORDER ISOPTERA									
KALOTERMITIDAE									
Kalotermes brouni						*			
ORDER PSOCOPTERA									
CAECILIIDAE									
Caecilius flavistigma	*	*				*			
Ectopsocus congener		*		*				*	*
MYOPSOCIDAE									
Myopsocus sp. P. 5.								*	
ORDER THYSANOPTERA									
AEOLOTHRIPIDAE									
Aeolothrips fasciatus	*	*	*			*			
THRIPIDAE									
? Anaphothrips obscurus T. 9	*	*	*	*		*			
Anaphothrips secticornis	*	*							
? Aptinothrips rufus T. 10		*	*			*			
Heliothrips haemorrhoidalis				*					
? Isoneurothrips obscuratus T. 11		*	*			*			
Thrips tabaci	*	*	*	*	*	*	*	*	*
? Thrips sp. T. 13		*	*		*	*			
PHLOEOTHRIPIDAE									
Haplothrips niger	*					*			
? Haplothrips sp. T. 2	*								
? Haplothrips corticis T. 3						*			

APPENDIX (Continued)

	Lucerne	Maize	Chou Moellier	Chou Moellier, Swedes, Mixed	Swedes	Turnips	Rape	Mangels	Rape, Swedes, Turnips, Mixed
ORDER NEUROPTERA									
HEMEROBIIDAE									
Micromus tasmaniae	*	*	*	*	*	*			
ORDER LEPIDOPTERA									
COLEOPHORIDAE									
Coleophora alcyonipenella	*								
PLUTELLIDAE									
Plutella maculipennis			*	*					
TINAEIDAE									
Choreutis bjerkandrella	*	*	*	*	*	*	*		*
Undetermined tinaeid Le. 10		*				*			
" " Le. 12	*		*	*	*	*		*	*
" " Le. 25	*								
TORTRICIDAE									
Austrotortrix postvittana	*	*	*	*				*	*
CRAMBIDAE									
Crambus flexuosellus	*	*	*			*	*	*	*
LYCAENIDAE									
Lycaena labradus	*	*	*	*	*	*			*
PIERIDAE									
Pieris rapae		*	*	*	*	*	*		*
GEOMETRIDAE									
Xanthorhoe ? aegrota. Le. 5	*	*		*		*			
ARCTIIDAE									
Nyctemera annulata				*					
NOCTUIDAE									
Agrotis ypsilon				*					
Heliothis armigera	*	*	*	*	*	*	*	*	*
Plusia chalcites	*	*	*			*			
Pseudaletia separata	*	*		*		*			

TECHNOLOGY

A PRECISION SEEDER OPERATED BY SUCTION

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(Received 7 September 1960)

Summary

A tractor-mounted precision seeder, for sowing single grains by means of suction applied to hypodermic needles, is described.

Introduction

The sowing of seeds singly by hand is one of the more tedious tasks at any plant breeding station, and the size of the populations grown for selection in early generations may be determined by the labour available, and not by the availability of material or land. The use of the precision seeder described in this paper has reduced the number of men required for the work, and has enabled a much greater area of single seeds to be sown.

The prototype of the suction-operated precision seeder (Sweetman 1957) was used as a guide in the development of the equipment. Several of the modifications suggested by Sweetman (*loc. cit.*) have been adopted, and further improvements were added during the construction.

The operation of the machine depends on the retention, by reduced air pressure, of individual seeds on the ends of small-bore tubes. These tubes are attached to a revolving reel, and the grains are lifted from the seed hopper and carried over to the horizontal position within the coulter. At this point the vacuum is interrupted, the seeds fall, and air at increased pressure passes through the tubes to remove dust. Provision is made for alteration of the spacing of the seeds in each row, and for variation in the gauge of the tubes for seeds of different crops.

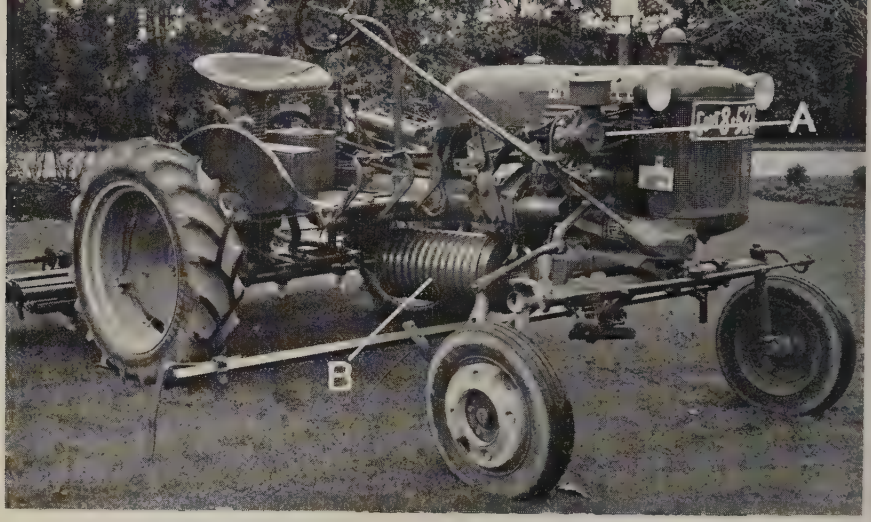


Fig. 1.—The tractor with all attached equipment. A—Hydraulic steering unit with drive from fan-belt. B—Vacuum tank from a hydraulic braking system.

Description of Seeder

The equipment is fitted to a commercial tractor, with additions as shown by the photographs (Figs. 1–6). A hydraulic steering unit (A—Fig. 1) from a different make of tractor is used to raise and lower the tool bar controlling the depth of sowing. The oil pump is driven from the fan belt. A vacuum tank (B—Fig. 1) and non-return valve (C—Fig. 2) from a hydraulic braking system, as fitted to commercial transport vehicles, are used to maintain an evenly low pressure in the pneumatic system. A vacuum pump (D—Fig. 2) with vacuum gauge from a small milking machine installation is driven from the power take-off (E—Fig. 2) of the tractor. The main drive (F—Fig. 2) for the selector reels is from the land wheel of the tractor, and variation of sprocket sizes allows for different seed spacing. An automatic clutch (G—Fig. 2) is fitted so that when the tool bar is lifted the sowers stop revolving. Row markers are fitted on both sides of the tractor.

Selector Reel

The selector reel (H—Figs. 4 and 6) is of steel shaft (diameter 2 in.), drilled to take the suction boss, and with eight holes drilled radially to take Luer-loc hypodermic needle adapters. Hypodermic needles, of a gauge to suit the seed to be sown, are shortened so that the distance from the tip of the needle to the shaft, including the needle holder, is a standard length (1½ in.). Needles of 16 gauge are suitable for wheat seed.

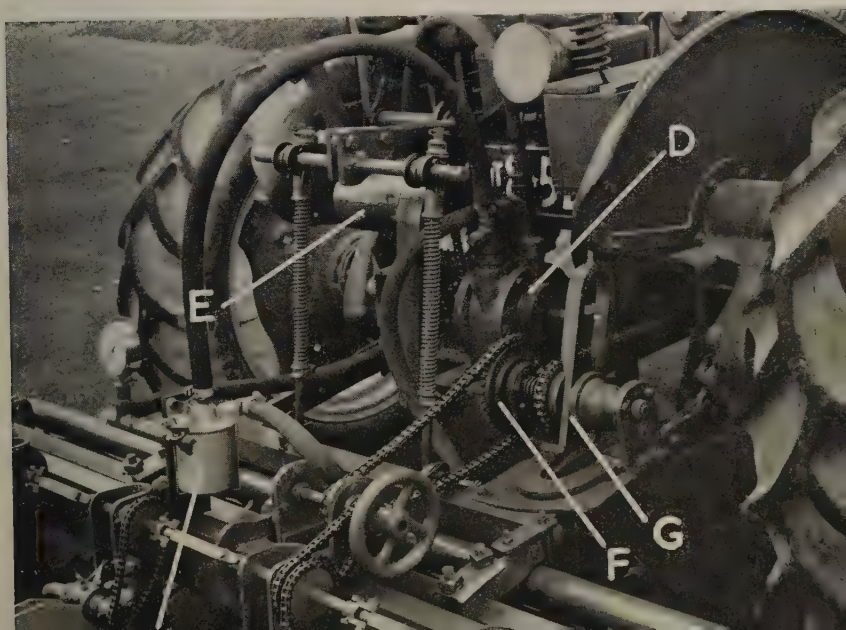
Suction Boss

The suction boss (M—Fig. 4) is of cast phosphor bronze with vacuum and pressure connections. A thin smear of grease provides a vacuum-tight joint between the stationary central boss and the revolving selector reel. When the machine is in operation, air at reduced pressure is drawn through the needles to the vacuum groove and thence to the vacuum pump (D—Fig. 2). Part of the air from the exhaust of the vacuum pump is passed between blocks in the vacuum groove, to each needle in turn, allowing the seed to drop, and clearing dust from the needle.

Adjustment for Suction Boss

A brass ring with lug is fitted at the outer end of the suction boss and attached to the coulter with a spring-loaded bolt. A set screw (N—Fig. 5) through the ring is used to position the suction boss so that air from the pressure tube passes through the individual needles as they reach the horizontal position within the coulter.

Fig. 2.—Rear of tractor with some of the attached equipment. C—Non-return valve from a hydraulic braking system. D—Vacuum pump from a milking machine installation. E—Power take-off from tractor. F—Drive for selector reels. G—Automatic clutch.



Seed Hopper

The seed hopper (O—Figs. 4, 5, and 6) is of cast aluminium in the form of a trough, hinged (P—Fig. 4) within the coulter. In the operating position, the sides of the hopper fit the selector reel (H—Figs. 4 and 6), and the hypodermic needles just clear the bottom. The end of the hopper within the coulter is sloped away from the selector reel, and the needles pass through a slit after the seed is dropped. This ensures that only those grains carried over by the needles are sown.

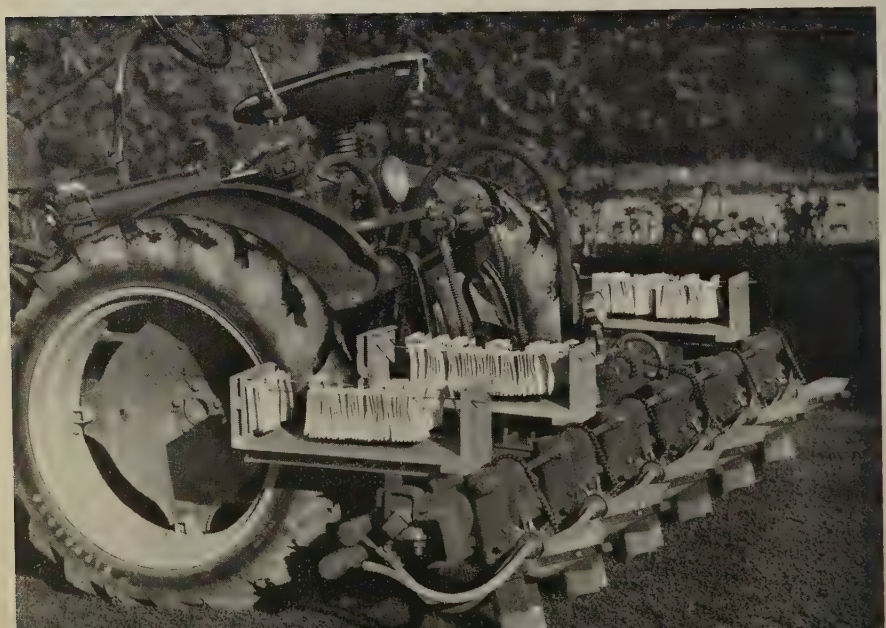
Coulter

The coulter (R—Figs. 4, 5, and 6) is of cast iron, with a tip similar to those of commercial drills, with bearings to take the top shaft, and with points of attachment to the tool bar. Provision is made for the attachment of the bearing of the selector reel, and the fitting for the variable adjustment (S—Figs. 4 and 5) and the quick-release (T—Fig. 6) of the seed hopper.

Manifolds

Two lengths of exhaust tubing serve as vacuum manifold (U—Fig. 4) and pressure manifold (V—Fig. 4). In the construc-

Fig. 3.—Racks fitted for holding wires and packets of seed for short rows.



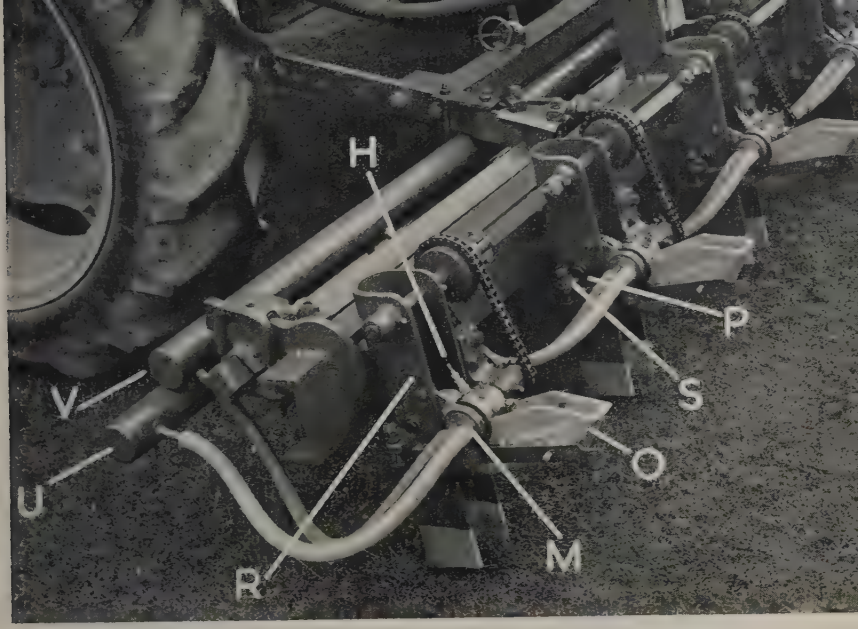


Fig. 4.—Tool bar and coulter assembly. H—Selector reel with Luer-locs and hypodermic needles. M—Suction boss with hose couplings to vacuum and pressure manifolds. O—Seed hopper hinged within the coulter. P—Hinge bolt. R—Coulter. S—Adjustment for seed hopper. U—Vacuum manifold. V—Pressure manifold.

tion, these were blocked at each end, and small pieces of tube were brazed in for connections to the sowers and the vacuum pump.

Connections

Rubber garden hose (1 in. diameter) is used for connections from the vacuum pump and vacuum tank to the vacuum and pressure manifolds. Thick-walled air hose ($\frac{3}{8}$ in. diameter) is used to make the connections between individual sowers and the vacuum and pressure manifolds.

Operation of the Sower

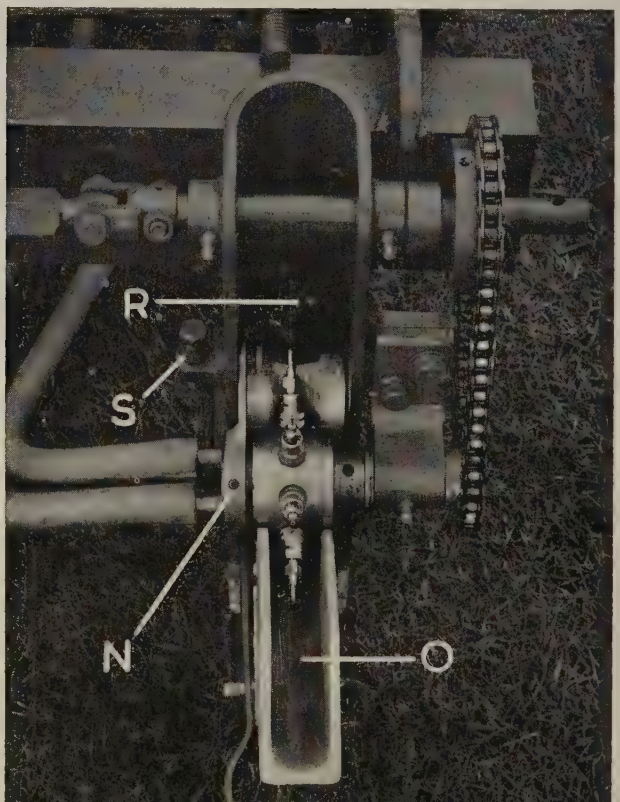
The majority of the field plots of the Cereal Section of the Crop Research Division, Department of Scientific and Industrial Research, Lincoln, are sown in rows 15 in. apart, for ease of inter-row cultivation. The sower is used for the early generations of wheat crosses from which single plants are selected. A cross in the F_2 generation is sown in a block of up to 18 rows, $2\frac{1}{4}$ chains long, containing approximately 8,000 plants.

In later generations a cross may be represented by several hundred single-plant progenies, sown in plots 4 ft long and either two rows or three rows wide. The seed for each short row is prepared beforehand and the packets threaded on wires held above the tool bar of the tractor (Fig. 3). During the sowing, the technician behind the sower takes the full packets of seed, empties them in the hoppers, and threads the empty packets on a spare wire. The tractor moves forward 5 ft, any surplus seed is emptied from the hopper, and the procedure is repeated.

The machine was developed to sow cereal grains singly, but during the construction it was realised that a wide range of seeds could be sown by this method, that similar equipment would be valuable at other plant breeding stations, and that it would be suitable for commercial sowings of fodder-beet and other crops which require thinning by hand.

Single and multiple precision sowers similar to the one described are now available.

Fig. 5.—Coulter assembly showing location of seed hopper. N—Locating screw for pressure tube. O—Seed hopper. R—Coulter. S—Adjustment for seed hopper.



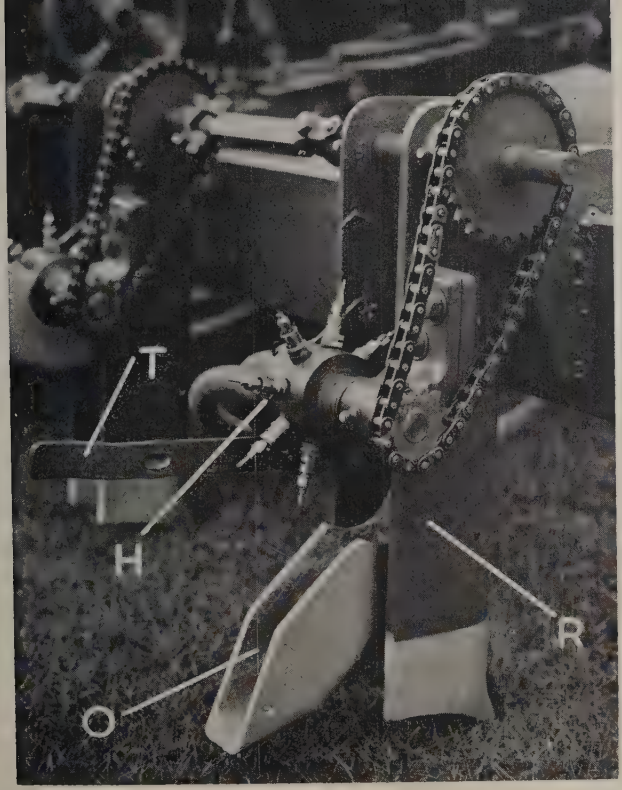


Fig. 6.—Coulter assembly with seed hopper lowered for cleaning. H—Selector reel. O—Seed hopper. R—Coulter. T—Quick release for seed hopper.

Acknowledgments

The assistance of Mr I. C. Sweetman, who loaned the prototype model, and of Mr D. C. Habgood and staff of Messrs W. A. Habgood Ltd is gratefully acknowledged. The photographs were taken by Mr A. P. Underhill.

Reference

Sweetman, I. C. 1957: A Suction-operated Precision Seeder. *N.Z. J. Sci. Tech. A38*: 577–82.

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